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A Summary of Current Program, 7/1/63;
and Preliminary Report of Progress
for 7/1/62 to 6/30/63

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NORTHERN

UTILIZATION RESEARCH AND DEVELOPMENT

DIVISION

of the

AGRICULTURAL RESEARCH SERVICE

UNITED STATES DEPARTMENT OF AGRICULTURE

This progress report of U.S.D.A. and cooperative research is primarily a tool for use of scientists and administrators in program coordination, development and evaluation; and for use of advisory committees in program review and development of recommendations for future research programs.

The summaries of progress on U.S.D.A. and cooperative research include some tentative results that have not been tested sufficiently to justify general release. Such findings, when adequately confirmed will be released promptly through established channels. Because of this, the report is not intended for publication and should not be referred to in literature citations. Copies are distributed only to members of Department staff, advisory committee members and others having a special interest in the development of public agricultural research programs.

This report also includes a list of publications reporting results of U.S.D.A. and cooperative research issued between July 1, 1962 and June 30, 1963. Current agricultural research findings are also published in the monthly U.S.D.A. publication, Agricultural Research. This progress report was compiled in the Northern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture, Peoria, Illinois.

UNITED STATES DEPARTMENT OF AGRICULTURE
Washington, D. C.
July 1, 1963

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INTRODUCTION

The Northern Utilization Research and Development Division, located at Peoria, Illinois, is one of four research divisions of the Agricultural Research Service concerned with the development of basic knowledge of chemical composition and physical properties of farm commodities and with the application of this knowledge to the development of new or improved products and processing technology that will enhance utilization of these commodities. The other Utilization Research and Development Divisions are the Eastern at Philadelphia, Pennsylvania, the Southern at New Orleans, Louisiana, and the Western at Albany, California.

The need and importance of utilization research on farm commodities arise from the fact that the farmer is not organized to carry on modern scientific research to maintain old, and create new, markets for his products. The Northern Division is responsible for utilization research concerned with industrial utilization of cereal grains, soybeans and flaxseed and with food and feed uses of corn, sorghum and soybeans. Responsibility for food and feed uses of wheat is assigned to the Western Division. In the Department's program of research on replacement crops the Northern Division conducts all screening and characterization studies on uncultivated plants and their components and is responsible for more intensive utilization research on new oilseeds containing erucic acid and on new gum and pulp fiber plants. Responsibility for detailed studies of additional selected new oilseeds is divided among the other three utilization research divisions. The Northern Laboratory also maintains small programs on sugarcane and forages. The major part of the Department's utilization research on sugarcane and forages is conducted at the Southern and Western Divisions, respectively.

In this report, utilization research of the Northern Division is discussed under the 11 Area Headings shown in the Table of Contents. For each area, a description of the current research program is provided, including domestic research contracts and sponsorship of related research performed abroad under grants or contracts involving PL 480 funds. A preliminary report of progress and a list of publications is given for each area for the period July 1, 1962 through June 30, 1963.

The scientific research effort at the Northern Division amounts to approximately 246 professional man-years. In addition, the Division supervises domestic research contracts equivalent to 28.5 professional man-years and sponsors a comprehensive program of research under PL 480. Following are some of the recent utilization research accomplishments of the Northern Division.

Cereal xanthide paper made on pilot-plant scale. By use of the process developed by Department researchers, paper containing cereal xanthide has been successfully produced on a pilot-sized paper machine. The water-insoluble cereal xanthides are produced by treating flours, starches, or

ground whole cereal grain with readily available, low-priced chemicals. Linerboard paper for corrugated paper boxes exhibited improved crush resistance under moist conditions, which is a major deficiency in conventional corrugated boxes. Bag paper containing 20 percent cereal xanthide exhibited superior wet and dry strength. Greaseproof paper containing 40 to 50 percent cereal xanthide showed improved properties over conventional greaseproof paper. Other applications are being developed. For example, wheat millfeed xanthide gives coarse, industrial paper with promising properties. Estimates indicate that the rapidly expanding paper products industry--now producing 37.5 million tons annually--could utilize cereal xanthides requiring over 100 million bushels of cereal grains per year.

Cationic dialdehyde corn starch in commercial production. Cationic dialdehyde starches have been developed by the Department's scientists that are excellent wet-end additives for increasing wet- and dry-strengths of paper without the use of retention aids as previously required with regular dialdehyde starch. Effective at low levels of application, they are less expensive to use, even though their unit manufacturing costs are slightly higher than regular dialdehyde starch. Practically all dialdehyde starch now consumed by industry for wet-end additives is of the cationic type. The Department's developments have served as a basis for and contributed to the methods used for all of the cationic dialdehyde starch now commercially manufactured. This new starch product has been proven to be economically competitive with synthetic chemical resins used as wet-strength additives.

Volume growth achieved in production of milky disease bacteria for control of Japanese beetle. Department research has demonstrated the feasibility of growing milky disease bacteria on a large scale in the vegetative form by submerged culture fermentation on grain-based media. The next step in development of this control agent for Japanese beetle is to obtain sporulation under industrially practicable conditions. So far, some success has been obtained in securing sporulation, but only on a solid medium. These and other studies have provided important new information on nutrients, bacterial metabolism, and previously unsuspected growth factors that should provide the basis for successful attainment of the objective. Work is continuing both in-house and by contract on all aspects of the problem.

Spores of milky disease bacteria induce the disease in Japanese beetle grubs and thus provide the best nonchemical agent now known for control of this serious plant pest. At present, spores must be obtained by the costly procedure of isolating them from infected grubs. Volume production of spores by industrially acceptable fermentation procedures would make this biological insecticide, which leaves no dangerous residues and is harmless to man and animals, an economical material for general use.

Enzymes convert corn and sorghum to inexpensive carbohydrate for industrial fermentations. Sirups obtained by enzymatic treatment of corn or sorghum are attracting intense industrial interest as economical replacements for imported molasses used in industrial production of chemicals and antibiotics by fermentation. In the process developed by Department scientists, starch in ground corn or sorghum is converted in high yield to glucose with a combination of bacterial and fungal amylases. After removal of solids, the resulting sirup is an inexpensive source of fermentable carbohydrate. On a sugar basis, cost of the sirup from corn is estimated to be at most equal to and probably less than that for high-test molasses or for blackstrap, if cost of cleanup by ion exchange is included. Steadily increasing prices and problems of availability are making molasses increasingly less attractive as a source of fermentable sugar. Adoption of the enzymatic process by the fermentation industry could lead to major expansion in the consumption of grain in this traditional market.

New protein food for developing countries. Cooperative studies between the Department, United Nations International Children's Emergency Fund (UNICEF), and industry have resulted in development of a simple, direct process for converting soybeans to full-fat soy flour, a nutritious protein-rich food. The process lends itself to the development of a compact truck- or trailer-mounted plant to process food for children in developing countries where large-scale industry is not yet feasible. Experimental lots involving several tons have been produced. The product has passed laboratory and animal feeding tests in this country, and UNICEF has made shipments to Taiwan and Indonesia for testing in children feeding programs.

Process improved for reducing the flavor unstable component of soybean oil. Department research has shown how the yield of soybean oil with enhanced flavor stability can be increased in the hydrogenation-winterization process. Yields of oil containing only 1 percent of the flavor unstable component (linolenic acid) amount to 75 percent if selective hydrogenation conditions are used and if stearine (high-melting) fractions are removed by use of acetone. Based on other research of the Department that showed linolenic acid to be the principal cause of flavor instability of soybean oil, industry introduced a process for its removal by hydrogenation followed by winterization to remove high-melting saturated and trans acids. Oils so processed have significantly improved stability, but for economic reasons it is important to recover as much liquid oil as possible. With the improved process developed by the Department, this result is accomplished and, in addition, the stearine fraction is upgraded and can be more readily marketed. Although hydrogenation-winterization is not a complete answer to the flavor problem of liquid soybean oil, it is a practical procedure that contributes significantly to increased use of soybean oil for salad and cooking purposes.

New chemical product from linseed oil attracts industrial interest. New cyclic fatty alcohols resulting from the Department's utilization research

on linseed oil are being evaluated for applications in the multimillion-pound market for long-chain fatty alcohols. Because of their unique cyclic structure, the new alcohols show unusual oil-like character and spreading properties not possessed by other long-chain alcohols of equal stability to oxidation. A major manufacturer is now testing cyclic linseed alcohols in its various products and is investigating their production for captive use and for sale to industry.

Potential of crambe as a new crop increased. Research on erucic acid, the principal fatty acid of crambe oil, and on processing crambe seed to produce palatable nutritious feed meal, has substantially increased the use potential for this new oilseed now under development in the Department's new crops research program. The practicability of conversion of erucic acid to dibasic brassyllic acid in good yield and purity has been demonstrated and promising results have been obtained in study and evaluation of this dibasic acid in several industrial end-uses. Concurrent studies on processing crambe seed to oil and meal have revealed significant new information on biologically important components of the meal and have provided the basis for new techniques for obtaining feed meal with acceptable nutritional qualities.

Department research has shown that crambe, a plant related to rape and mustard, has excellent crop potential and gives satisfactorily high yields under dry-farming and irrigation conditions. It can be grown in place of crops now in over-abundant supply and in areas where there is no locally grown oilseed meal crop. Crambe oil would compete with imported rapeseed oil but not with presently grown domestic vegetable oils.

AREA NO. 1: CEREAL STARCHES INDUSTRIAL UTILIZATION

Problem. Starch accounts for about two-thirds the weight of all grains. Finding new, large-volume outlets for starch would, therefore, result in substantially increased consumption of cereal grains. Of the 4.5 billion pounds of cereal starch now produced, about 2.7 billion pounds is used ultimately in food products, and increases would be expected to follow population growth. However, the remaining 1.8 billion pounds find industrial outlets that offer opportunities for increases at a rate greater than that of population growth. Because starch must compete with products derived from nonagricultural sources, these opportunities can best be realized by a program of research designed both to maintain the competitive position of starch in its current uses and to develop economical new industrial uses.

New outlets for cereal starches and flours equivalent to over 200 million bushels of grain by 1975 can be envisioned if basic research and development on several types of chemical and physical modification of starch and flour now in the experimental stage or anticipated can be prosecuted to successful conclusion. About 150 million bushels could be required for new products for the pulp and paper industries and for the building and construction industries, and about 10 million bushels each for other industries such as the chemical, petroleum, mining, textile, plastics, coatings, and packaging industries. New outlets for starch that appear very promising include use of modified starches as wet-strength additives for paper, water-resistant adhesives, coatings, and foamed products, and of starch-derived pulps as an integral part of high-quality paper. In addition, if the competitive position of starch is successfully maintained through improvement by research, additional consumption would be expected by 1975 from participation in markets for 100 million bushels of grain resulting from normal growth of existing industrial outlets for starch and flour such as paper, textiles, packaging adhesives, drilling muds, and building materials.

To accelerate realization of these goals, more information is needed on the physical and chemical properties and chemical reactions of cereal starches, on economical methods for effecting desired physical and chemical modifications and on product evaluation and development. In addition, still further new markets for cereal starches should be possible from an adequate program of fundamental and exploratory research to discover new concepts, principles, and reactions leading to new processes and products for future development.

USDA PROGRAM

The Department conducts a continuing, long-range program of research involving analytical, organic and physical chemists and chemical engineers engaged in basic, applied and developmental studies on the chemistry of cereal starches and their conversion to useful industrial products.

The Federal scientific effort for research on cereal starches totals 55.0 professional man-years. Of this number 11.0 are devoted to chemical composition and physical properties and 44.0 to new starch chemical derivatives and their evaluation.

Research at Peoria, Illinois, on chemical composition and physical properties (9.2 professional man-years) involves fundamental research on reactions of starch and dextrose in nonaqueous solvents. Research contracts under this subheading (1.8 professional man-years) are in effect with the University of Arizona, Tucson, Arizona, for basic studies on the reaction of starch with mercaptans (.6 professional man-year) and with acetylene (.5 professional man-year); and with The Johns Hopkins University, Baltimore, Maryland, for basic research on the reactions of starch in fluid dynamic media (.7 professional man-year).

Research at Peoria, Illinois, on new starch chemical derivatives and their evaluation (32.6 professional man-years) involves basic and applied studies on various types of chemical products derived from starch and dextrin and in evaluation of these products for various industrial uses such as pulp and paper products, plastics, coatings, organic chemicals and stable viscosity agents. During the reporting period research was discontinued on production of hydroxymethyl furfural from starch and on use of dialdehyde starch as a raw material for new chemical products. Research contracts under this subheading (11.4 professional man-years) are in effect with the University of Minnesota, St. Paul, Minnesota, for studies on reactions of dialdehyde starch in solution (.4 professional man-year); with Ohio State University, Columbus, Ohio, for research on synthesis of amino derivatives of starch (1.2 professional man-years); with the State University of New York, Syracuse, New York, for evaluation of crosslinked hypochlorite-oxidized starches in papermaking (.3 professional man-year); with Battelle Memorial Institute, Columbus, Ohio, for evaluation of allyl dialdehyde starch in coatings and resins (2.0 professional man-years) and for developmental research on starch and other cereal grain xanthides (6.2 professional man-years); and with Stanford Research Institute, Menlo Park, California, for research on graft copolymers of cereal starches with vinyl-type monomers (1.3 professional man-years).

The Department also sponsors research on cereal starches conducted by foreign institutions under grants of PL 480 funds. Research on chemical composition and physical properties involves grants to the Institute of Fibres and Forest Products, Jerusalem, Israel, for research on hypohalite

oxidation of cereal starches (5 years, 1958-1963); University of Birmingham, England, for research on starch structure as revealed by interaction of starch and enzymes (5 years, 1959-1964); National Institute of Hygiene, Paris, France, for research on proteolysis inhibiting effects of cereal starches and flours (3 years, 1961-1964); National Institute of Agronomic Research, Paris, France, for research on changes induced in starch by gamma irradiation (4 years, 1961-1965); and Scientific Institute for Chemistry and Biochemistry, Milan, Italy, for research on glucopyranose rings in starches and dextrins (5 years, 1962-1967). New starch chemical derivatives and their evaluation involves grants to the Arthur D. Little Research Institute, Musselburgh, Scotland, for research on glucose-derived polymers (4 years, 1959-1963); Hebrew University, Jerusalem, Israel, for studies of methods for preparing fluorine derivatives of starch (3 years, 1961-1964); Institute of Industrial Chemistry, Bologna, Italy, for studies on fatty chemical derivatives of starch dextrins (5 years, 1960-1965); National Institute of Technology, Rio de Janeiro, Brazil, for research on phosphorus- and sulfur-containing cationic starches (5 years, 1962-1967); and Ahmedabad Textile Industries Research Association, Ahmedabad, India, for research on starch-gum copolymers prepared by codextrinization (5 years, 1963-1968).

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition and Physical Properties

1. Reactions of starch and dextrose in nonaqueous solvents. Yields of pure disorbitylamine hydrochloride have been increased to 64 percent of theory. The procedure was shown to be generally applicable to aldoses. Preliminary cost estimates for disorbitylamine suggest a selling price of about 50 cents per pound (without provision for recovery and sale of by-products). A survey of market potential conducted by the ERS representative at NU indicated a potential market for organic chelating agents of about 30 million pounds per year. In view of its probable selling price, disorbitylamine appears to merit further consideration for end uses such as textile sequestrants and biodegradable detergents. Derivatives of disorbitylamine of particular interest are those combining sequestration and surface activity. Another product, isomaltol, has attracted the attention of a commercial firm, which has indicated intention to produce this product under NU patents.

Further study of complexes of carbohydrates and alkali metal hydroxides showed that in the nonaqueous solvent, N-methyl-2-pyrrolidone, such complexes are at least 85 percent in the alcoholate form, whereas when these complexes are formed in aqueous media, they are not alcoholates but rather simple adducts. Studies on formation of complexes of alkali metal salts and carbohydrates in alcoholic media have clarified the effects of salt concentration and of the presence of water. Complexes of the metal salt with two carbohydrate molecules are favored by anhydrous conditions and

low salt concentration. With higher salt concentrations and up to 5 percent of water, the ratio of salt to carbohydrate in the complex increased. In the presence of water, alkali hydroxides behaved like salts; e.g., complexes ranged from 1 sucrose.3 NaOH to 2 sucrose.1 NaOH.

2. Reactions of starch with acetylene and mercaptans. In contract research at the University of Arizona, statistical analysis of experimental data revealed optimum conditions for five variables (time, temperature, pressure, proportion of KOH, and solvent ratio) in the reaction of acetylene with starch.

Starches were observed to differ in reactivity to acetylene. For best results corn and sorghum starches required an activation treatment which was not necessary for wheat, waxy corn or waxy sorghum, high-amylose corn starch, potato starch and corn amylose. Vinyl starches of 0.6 to 1.1 degree of substitution were soluble in methanol, acetone, dioxane and tetrahydrofuran but not in water, ether, benzene and carbon tetrachloride. Films from polymers of vinyl amylopectin and of vinyl high-amylose corn starch were brittle.

Other contract studies at Arizona on glucose-mercaptop reaction products yielded dark-colored materials showing some promise as adhesives.

3. Reactions of starch in fluid dynamic systems. Early results of contract studies at Johns Hopkins University indicate that reactions of starch with propylene glycol or dimethylsulfoxide at temperatures above 100° C. merit further study in fluid flow systems.

4. Hypohalite oxidation of starches. At the Institute for Fibres and Forest Products Research, Jerusalem, Israel, a chemical equation has been developed on the basis of kinetic data that describes the oxidative reaction, and the influence of different process conditions on the rate of reaction has been determined. Analytical determinations on oxidized starches have revealed the amount of starch polymer cleavage and the number and type of new functional groups that result from oxidations under different conditions. This work is providing a basis for the correlation of chemical structure with useful physical and chemical properties so that products of uniform quality can be routinely made, and so that products of improved quality for specific end uses can be obtained by altering process conditions. This research, which was performed under a PL 480 grant, has been completed.

5. Starch structure. In studies conducted by scientists at the University of Birmingham, England, under a PL 480 grant, reversible reactions between large molecules are being investigated by sedimentation and electrophoresis methods with the aim of interpreting the interaction of starch with its associated enzymes. Procedures were developed for successful enzymatic synthesis of model starch molecules of exceptionally uniform molecular

weight distribution. At the "Giuliana Ronzoni" Scientific Institute of Chemistry and Biochemistry, Milan, Italy, studies under a PL 480 grant on amylose deuteration have confirmed that the hydroxyl hydrogen atoms of amylose are exchanged completely with deuterium. Tentative assignment of infrared absorption bands for freeze-dried cyclodextrins, amyloextrins, and amylose has been made.

6. Proteolysis inhibition by starch. A water-soluble, heat-labile, trypsin inhibitor was found in wheat flour as well as in corn, barley, rye, oats, rice, millet, and buckwheat. No inhibitor was detected in wheat bread, and only a small amount in rye bread. Starches showed no inhibiting activity, although their adsorption of proteolysis products caused some interference in the application of the assay method which was developed. This research is being performed by the National Institute of Hygiene, Paris, France, under a PL 480 grant.

7. Effects of γ -radiation on starch. Results of studies now in progress under a PL 480 grant at the National Institute of Agronomic Research, Paris, France, indicate (1) that glucose units are so altered by γ -irradiation that they no longer can yield 5-hydroxymethyl furfural; (2) that with increased irradiation more starch becomes soluble in water and the fraction of starch comprising chains of less than 14 glucose units increases; (3) that susceptibility to the action of α -amylase increases; and (4) that increased amounts of ionizable groups are formed. These results are consistent with the interpretation that γ -irradiation causes chemical changes at OH groups, ruptures starch chains, breaks hydrogen bonds and alters the starch granule membrane. The water content during irradiation appears to play a leading role in the development of these changes.

B. New Starch Chemical Derivatives and Their Evaluation

1. Studies on starch xanthates and xanthides. Studies on starch xanthates and xanthides (called "cereal pulps" in last year's report) are establishing a sound basis for development of a practical process for their preparation and use in paper. A semicommercial-scale trial run was successfully performed at Forest Products Laboratory in which an unfilled bond-type paper containing 12.5 percent of starch xanthide was produced. Dry tensile and bursting strengths of the product were 60 percent and 120 percent greater than the respective values for the control. Wet strength was increased 500 percent and brightness decreased about 10 percent. Feasibility of large-scale use has thus been demonstrated, and several aspects of such use requiring special attention, such as pH control, mixing, and odor problems, have been identified.

Investigations on the conversion of cereal xanthate to xanthide with hypochlorite have revealed conditions for reducing the oxidant requirement from 1.5-2.0 moles to about 1.1 mole per xanthate group. Conditions have also been found for efficient use of hydrogen peroxide as the oxidant. No

metal ion catalyst is needed and the oxidant requirement is close to theory. An automatic system for controlling and recording pH and redox potential during oxidative coupling of xanthate was devised.

Xanthates may be added in solution to a wood pulp suspension and then crosslinked to insoluble xanthides (in situ formation) or they may be separately insolubilized and added to the pulp (ex situ formation). In laboratory studies, addition of xanthide prepared ex situ gave variable results. However, exploratory experiments have provided leads to techniques that may result in consistently good wet and dry strength increases by ex situ formation of xanthide. In use of xanthide formed in situ, freshness of xanthate and presence of alum favorably influenced retention. Wet strength of xanthide papers was improved by heating or extended low-temperature equilibration.

A contract, covering development of a practical process for continuous production of cereal xanthate and for optimum use in paper of xanthide formed ex situ, has been negotiated with Battelle Memorial Institute.

2. Evaluation of dialdehyde starch (DAS) and derived products. Extensive evaluation studies on DAS-betaine hydrazones (cationic DAS) showed that 2.5 percent of cationic DAS produced a wet-strength increase equal to that obtained with 2.5 percent each of ordinary DAS and cationic-starch retention aid. Addition of more than 2.5 percent of cationic DAS gave greater increases than were possible with DAS plus retention aid. All of the desirable properties imparted to paper by DAS plus retention aid are also imparted by cationic DAS. Simultaneous cationization and dispersion of DAS were readily achieved by heating briefly at 92° C. a suspension of DAS to which the betaine was added. Large-scale trials of cationic DAS in bag paper and toweling were made at Forest Products Laboratory. The results were very successful. Retentions of 84-90 percent of the cationic DAS were achieved.

Birch plywood adhesives prepared by reacting casein and DAS had rapid cold set and good dry strength. Dry shear strength and wet strength (after 48 hours soaking in cold water) exceeded values for commercial casein glue. The DAS-casein glue bond also resisted delamination in boiling water. However, poor bonding was obtained with Douglas fir, presumably because penetration was decreased by the resins present in the wood. Light sanding was found to improve greatly adhesion with Douglas fir, and preliminary tests of isopropanol as an additive to the glue to promote penetration appeared promising. For this development to achieve success it will be necessary to solve the problem of adhesion to Douglas fir, which represents the major portion of the potential market. In 1960, about 270 million pounds of adhesives were used by the plywood industry. Casein adhesives, if crosslinked by DAS, may be suitable for exterior use. Such use would represent a substantial new market for DAS. Present results also indicate

that DAS has potential for improvement of animal blood and soy flour adhesives, which together make up the bulk of the 120 million pounds of protein adhesives now used in plywood.

Studies at the University of Minnesota were continued on partially oxidized corn starch in an effort to determine the mode of attack of periodate on starch. Borohydride reduction with subsequent hydrolysis of 29 percent and 43 percent periodate-oxidized starch yielded glycerol, erythritol, 2-O- α -D-glucopyranosyl-D-erythritol, and a series of erythritol glucosides of higher oligosaccharides. Two different disaccharide fractions were also obtained which are being characterized.

At Battelle Memorial Institute, allyl DAS was found to have the solubility and viscosity properties and compatibility with plasticizers required for commercial lacquers. Films cast on tin plate and cured for 30-60 minutes at temperatures from 140° to 400° F., were hard but had inadequate strength when unsupported. A molded product obtained by copolymerization of allyl DAS and polyethylene glycol dimethacrylate had promising properties. Studies on chemicals from DAS were discontinued, except for preparation of materials for use in the contract work at Battelle.

In contract research at the State University of New York, hand sheets were prepared with two levels of addition of dialdehyde wheat starch, 90-93 percent oxidation, and dialdehyde corn starch, 10 percent oxidation. Tests of the products as wet end additives are, however, not yet complete.

Standard dress shoes with soles of experimental DAS-tanned leather (prepared during contract research by Armour Leather Company) were manufactured according to Quartermaster Corps specifications in September 1962. These were cross-matched with standard dress shoes and are being given service tests at the Quartermaster Field Evaluation Agency at Fort Lee, Virginia. A final report on the performance of the experimental shoes should be available by the middle of 1964.

3. Chemical products from starch and dextrin. Several new acetals of starch, amylose and glucose were prepared. They showed a very wide range of properties depending on the carbohydrate and the degree of substitution. Low D.S. (degree of substitution) starch-dihydropyran acetals appeared to have good properties as warp sizes for synthetic yarns.

So far, attempts to improve the coagulant properties of starch by introducing differing amounts of acrylamide groups into cationic starch have not been successful. Very low D.S. (below 0.1) cyanoethylated starches and dextrans had promising properties as paper-coating adhesives and gave better bonding than commercial starch adhesives for coatings containing low (40 percent) solids content.

Polyol glycosides (from reaction of starch and a glycol) were polyetherified by reaction with propylene oxide. Rigid urethane foams, made from the

polyethers by the prepolymer method, had densities of 1.7 to 2.1 pounds per cubic foot and dimensional stability and compressive strengths sufficiently good to justify more extended study and evaluation. Rigid foams based on polyethers of starch polyol glycosides appear to have commercially acceptable properties. Cost estimates indicate that they could be produced at competitive prices. Since the world market for rigid foams is expected to exceed 110 million pounds by 1965, success in this development could lead to a significant new market for starch.

In contract research at Ohio State University, several aminated amylose derivatives have been prepared.

At the Hebrew University, Jerusalem, Israel, several model compounds such as β -tetraacetyl glucose-6-bromohydrin have been prepared and exchange reactions between the halogen in these models and various inorganic fluorides are being studied to provide guidelines for introducing fluorine into starch. In studies at the National Institute of Technology, Rio de Janeiro, Brazil, starch was reacted with thioglycolic acid in the presence of sulfuric acid catalyst to obtain starch thioglycolate. This product was reacted with ethyl iodide to yield the sulfonium derivative which appeared to have cationic properties. Scientists at the Institute of Industrial Chemistry, Bologna, Italy, have made many fatty ester and some fatty amine derivatives of fractions of corn dextrin. The results are promising for locating a possible source of biodegradable detergents utilizing waste fats and surplus cereal grains. These studies are being conducted under PL 480 grants.

4. Evaluation of starch derivatives in paper and paper products. Research on new chemical products from starch is supported by evaluation studies to determine the quality and performance of these products in applications in the pulp, paper and paperboard industry. During the reporting period tests were conducted, for example, with starch xanthates and xanthides, cationic dialdehyde starch, and cyanoethylated starch. Results of the evaluation studies are reported in conjunction with the general discussion of research on the specific starch product.

5. Polymers based on carbohydrates. Investigators at the Arthur D. Little Research Institute, Musselburgh, Scotland, have prepared a new water-soluble glucose polymethacrylate as well as the first known high molecular weight polymer of a glucose-derived vinyl ether. Other novel polymeric products synthesized include a series of polyphenyl esters derived from bisphenol A and carbohydrate diacid chlorides and a series of nylon-6,13-type polyamides obtained by reaction of brassyloyl chloride and carbohydrate diamines. Several of the nylon-6,6-type polymers reported previously are being evaluated by British industrial firms for use in fibers and as adsorbents for removal of protein tannin haze in beer. This work is being conducted under a PL 480 grant.

PUBLICATIONS AND PATENTS REPORTING RESULTS
OF USDA AND COOPERATIVE RESEARCH

Chemical Composition and Physical Properties

Gilbert, L. M., and Gilbert, G. A. (University of Birmingham, England). 1962. Sedimentation of β -lactoglobulin A under aggregating conditions. *Nature* 194(4834), pp. 1173-1174.*

Hodge, J. E., and Fisher, B. E. 1963. Amadori rearrangement products. In "Methods in Carbohydrate Chemistry." Edited by R. L. Whistler, Vol. 2, pp. 99-107.

Hodge, J. E., and Nelson, E. C. Sept. 18, 1962. Process for preparing isomaltol. U. S. Patent 3,054,805.

Hodge, J. E., Nelson, E. C., and Moy, B. F. 1963. Metal chelation by glucose-ammonia derivatives. *J. Agr. Food Chem.* 11(2), pp. 126-129.

Mejzler, D., Schmorak, J., and Lewin, M. (Institute for Fibres and Forest Products Research, Jerusalem, Israel). 1962. Estimation of the number average DP of starch by the periodate method. *Die Stärke* 14(11), pp. 428-432.*

Schmorak, J., and Lewin, M. (Institute for Fibres and Forest Products Research, Jerusalem, Israel). 1962. The properties of wheat and waxy maize starches mildly oxidized with sodium hypochlorite. *Bull. Res. Council Israel, Sect. 11A*(3), pp. 228-229.*

Schmorak, J., Mejzler, D., and Lewin, M. (Institute for Fibres and Forest Products Research, Jerusalem, Israel). 1962. A study of the mild oxidation of wheat starch and waxy maize starch by sodium hypochlorite in the alkaline pH range. *Die Stärke* 14(8), pp. 278-290.*

Van Cleve, J. W. 1963. D-fructofuranose 1,3,4,6-tetrabenoate. Direct benzoylation of D-fructose. In "Methods in Carbohydrate Chemistry." Edited by R. L. Whistler, Vol. 2, pp. 237-238.

New Starch Chemical Derivatives and Their Evaluation

Bird, T. P., Black, W. A. P., Dewar, E. T., and Hare, J. B. (Arthur D. Little Research Institute, Musselburgh, Scotland). 1963. Polyamides containing carbohydrate residues. *J. Chem. Soc.* 1963, pp. 1208-1212.*

Black, L. T., Lancaster, E. B., and Maister, H. G. 1963. Determination of chromium and lead in periodic acid solution and dialdehyde starch. *Cereal Chem.* 40(1), pp. 66-71.

Black, W. A. P., Dewar, E. T., and Rutherford, D. (Arthur D. Little Research Institute, Musselburgh, Scotland). 1962. A polyvinyl ether containing sugar residues. *Chem. & Ind. (London)* 1962(36), p. 1624.*

Ernst, A. J., Carr, M. E., Weakley, F. B., Hofreiter, B. T., and Mehltretter, C. L. 1962. Dialdehyde starch-casein paper coating adhesives for improved wet-rub resistance. *Tappi* 45(8), pp. 646-650.

Gugliemelli, L. A., Mayer, G. L., and Russell, C. R. Nov. 13, 1962. Allylated dialdehyde starch. U. S. Patent 3,063,855.

*Research supported by PL 480 funds.

Hofreiter, B. T., Hamerstrand, G. E., and Mehlretter, C. L. Nov. 6, 1962.
Wet-strength paper containing polymeric dialdehydes. U. S. Patent
3,062,703.

Hofreiter, B. T., Hamerstrand, G. E., and Mehlretter, C. L. Dec. 4, 1962.
Process for making high wet-strength paper containing polymeric dialdehyde. U. S. Patent 3,067,088.

Hofreiter, B. T., Hamerstrand, G. E., and Mehlretter, C. L. Apr. 30, 1963.
Cationic polymeric dialdehydes and their use in making wet strength paper.
U. S. Patent 3,087,852.

Kay, D. J., Hamerstrand, G. E., and Hofreiter, B. T. 1962. Rapid photometric determination of dialdehyde starch in paper. *Tappi* 45(12), pp. 943-944.

Mehlretter, C. L., Yeates, T. E., Hamerstrand, G. E., Hofreiter, B. T., and Rist, C. E. 1962. Preparation of cationic dialdehyde starches for wet-strength paper. *Tappi* 45(9), pp. 750-752.

Naffziger, T. R., Hofreiter, B. T., and Rist, C. E. 1962. Upgrading insulating board and molded pulp products by minor additions of dialdehyde starch. *Tappi* 45(9), pp. 745-750.

Otey, F. H., Mehlretter, C. L., and Rist, C. E. 1963. Polyoxyethylene ethers of some polyol glycosides and their fatty esters. *J. Am. Oil Chemists' Soc.* 40(2), pp. 76-78.

Otey, F. H., Wilham, C. A., and Mehlretter, C. L. July 24, 1962.
Process for making erythritol and ethylene glycol from polymeric dialdehydes. U. S. Patent 3,046,312.

Schaefer, W. C., Maurice, J. J., Russell, C. R., and Rist, C. E. 1962.
Intrinsic viscosity of dialdehyde starch in alkaline urea after reduction with borohydride. *Cereal Chem.* 39(4), pp. 304-310.

Schaefer, W. C., Russell, C. R., Maurice, J. J., and Rist, C. E. 1963.
Thin-boiling starches from the reaction of corn starch with chlorine in methanol. *Cereal Chem.* 40(2), pp. 101-109.

Wagoner, J. F.,¹ Stemoroski, J. C.,¹ Windus, W.,² and Witham, W. C.
(¹Armour Leather Co., Williamsport, Pa.; ²Eastern Util. Res. Devlpmt. Div., Agr. Res. Serv., U.S. Dept. Agr., Philadelphia, Pa.). 1962.
Dialdehyde starch as a pretanning agent for vegetable-tanned sole leather. *J. Am. Leather Chemists' Assoc.* 57(7), pp. 302-317.

Weakley, F. B., Ashby, M. L., and Mehlretter, C. L. 1963. Casein-dialdehyde starch adhesive for wood. *Forest Prod. J.* 13(2), pp. 51-55.

AREA NO. 2: WHEAT INDUSTRIAL UTILIZATION

Problem. Wheat traditionally commands a higher price than corn. Since the starch content and starch properties of these two cereals are similar, new industrial uses for wheat must rely on advantages to be obtained from other components. Wheat flour is a mixture of starch, protein, gums, fiber, and fat. Because of the simultaneous presence of these basic ingredients, opportunities are promising for development of a wide variety of industrial products from wheat flour that would be expected to have properties and uses different from those of related products derived from refined starch. The problem is to find means for economical modification and reaction of these ingredients with each other and with other chemicals in order to realize the potential of the combinations.

Basic research now being conducted by the Department points to new potential industrial uses for cereal starches and flours that could consume over 200 million bushels of grain by 1975. Among potential outlets for wheat flour are sizes for many special grades of paper, cereal pulps that would form an integral part of such papers, and plastic or foamed compositions for hardboard and insulating boards. The opportunity for successful realization of these possibilities is enhanced by recently developed fine-grinding and air-classification milling techniques that permit the composition of flour to be varied over wide ranges. These techniques are now satisfactory for soft wheats, but ways must be found to adapt them to hard wheats which constitute 93 percent of the wheat remaining after current needs have been met.

Wheat flour could achieve its share of potential new markets more rapidly, and discovery of additional new uses under both public and private research programs would be facilitated, if more information were available on the basic physical properties and chemical reactions of flour and its components, on tempering and milling techniques, and on processing methods for economical conversion of flour to desired end products.

USDA PROGRAM

The Department conducts a continuing long-range program of research involving analytical, organic and physical chemists, chemical engineers and structural biologists engaged in basic studies of the chemical and physical properties of wheat, flour, flour fractions, and protein components and in applied research leading to new and improved wheat products for industrial use.

The Federal scientific effort for research on industrial utilization of wheat totals 47.2 professional man-years. Of this number 14.4 are devoted

to chemical composition and physical properties; 20.6 to industrial chemical products; and 12.2 to processing technology.

Research at Peoria, Illinois, on chemical composition and physical properties (12.0 professional man-years) includes separation, characterization and chemical reactions of the component proteins of wheat gluten. Research contracts (2.4 professional man-years) are in effect at Purdue University, Lafayette, Indiana, for fundamental studies of the alkaline desulfurization of gluten (.8 professional man-year); and Armour Research Foundation, Chicago, Illinois, for investigation of methods for controlled hydrolysis of gluten (1.6 professional man-years).

Investigations on industrial chemical products conducted at Peoria, Illinois, (17.5 professional man-years) involve preparation and evaluation of new types of water-soluble and water-insoluble flour derivatives for industrial use. During the reporting period laboratory studies on hydrophilic flour derivatives, such as sulfated wheat flour, was placed in abeyance to permit strengthening of the research effort on xanthates and xanthides of starch, wheat flour and other cereal products. Research contracts (3.1 professional man-years) are in effect with Stanford Research Institute, Menlo Park, California, for basic research on graft copolymers from wheat flour and starch (1.4 professional man-years); and Iowa State University, Ames, Iowa, for studies on development of improved adhesives from wheat gluten by reaction with dialdehyde starch (.8 professional man-year) and for engineering studies on use of pneumatic fluidization to effect acid modification of flour (.9 professional man-year).

Processing technology research at Peoria, Illinois, (11.6 professional man-years) involves studies on conditioning and milling of wheat, air classification of flours, and reduction of viable microorganisms in wheat flour. A research contract (.6 professional man-year) with Kansas State University, Manhattan, Kansas, is concerned with study of the mechanism of enzyme formation during wheat malting and relationship of the information developed to control of enzymes and their action during milling and processing of wheat.

The Department also sponsors research in this area conducted by foreign institutions under grants of PL 480 funds. Research on chemical composition and physical properties involves a grant to the Weizmann Institute of Science, Rehovot, Israel, for synthesis and study of polypeptides having amino acid compositions related to wheat gliadin and corn zein (4 years, 1960-1964). Research on processing technology involves a grant to the Research Association of British Flour Millers, St. Albans, England, for investigations on quantitative measurement of properties of wheat that change significantly during conditioning (4 years, 1961-1965).

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition and Physical Properties

1. Characterization of wheat gluten proteins. Titration studies on gluten showed that only about 10 percent of the amino acid residues contributed ionizing groups. These groups, in descending order of frequency of occurrence, were carboxyl, tyrosyl, imidazole, lysyl, α -amino and sulphydryl. The ionization constants of these groups were all normal, showing absence of interaction or structural anomalies in dilute solution. Guanidyl groups were not titrated since the pK of this group is greater than 13. By chromatography, reduced-alkylated glutenin was separated into three fractions, each containing two of the six major components present in reduced glutenin. Amino acid analysis of the three chromatographic fractions revealed generally similar compositions, with the major portion (ca. 70 percent) of the residues accounted for by glutamine, proline, and glycine. Significant differences between the fractions were observed in the content of arginine, histidine, alanine, valine, and phenylalanine. These differences establish that glutenin is composed of several distinct polypeptide chains.

The molecular weights of native and reduced gamma-gliadin were obtained by sedimentation analysis in 4 M guanidine hydrochloride. A molecular weight of 26,000 was obtained for both the native and reduced proteins, indicative of the single chain structure of gamma-gliadin. This is in agreement with the observation of only one component after starch gel electrophoresis of reduced protein. A minimal molecular weight of 25,000 was calculated from the amino acid analysis of the gamma component. Further characterization of gamma-gliadin showed that aspartic acid occupies the N-terminal position and serine the C-terminal position. A probable sequence in the C-terminal portion of gamma-gliadin isLeu . Thr . Gly . Ser.COOH.

Information on aggregation, ionic interaction, solubility, structure, reactions and other physical and chemical properties of the gluten proteins is basic to understanding and control of properties and to chemical modification of gluten and derived products, as such or in flour. Research during this period has made important new contributions to such fundamental information.

2. Chemical reactions of wheat gluten. Water-soluble, synthetic polypeptides containing glutamine residues were insoluble in water at low pH unless a small amount of urea was present. Since the polypeptides retained helical formation in the urea-containing solutions, only side-chain amide associations appear to have been disrupted. Solubility of gliadin in solutions at increased ionic strength was greatly decreased by reduction and alkylation. The same treatment did not affect glutenin significantly. Configurational changes in gliadin may account for this difference.

In studies of model compounds, the rate of reaction at pH 8.3 of acrylonitrile with the sulphydryl group of mercaptopropionic acid was 74 times

more rapid than the rate of its reaction with the amino group of β -alanine. Cyanoethylation of a mixture of these compounds resulted in complete reaction of SH groups accompanied by reaction of 10 percent of the NH₂ groups. Reoxidation in dilute solution of either reduced glutenin or gliadin gave a final product resembling the original gliadin. In concentrated solution, cohesive particles soluble only in urea-bisulfite solutions were obtained.

The research on cyanoethylation of sulphydryl and amino groups is part of a broader study designed to throw light on the kinetics and other characteristics of reactions of vinyl compounds with selected chemical groups of amino acids found in wheat protein. These reactions are important to chemical modification of protein for industrial purposes. The information obtained in this study, as well as that resulting from characterization of individual protein components and from other basic research on composition and properties of wheat protein, is essential to the development of new products from either wheat protein, flour, or flour fractions.

In contract research at Armour Research Foundation, hydrolysis of gluten in formic, trichloracetic or hydrochloric-acetic acid solutions yielded products shown by sedimentation to be nonuniform in molecular size. At Purdue University, contract research on desulfurization has been concerned with establishment of methodology and preliminary experimentation.

3. Synthetic polypeptides related to wheat gliadin. Modified gliadins soluble in water at neutral pH were prepared by grafting either polyalanyl or polyaspartyl side chains to the gliadin. The solubility of wheat gluten in water was enhanced considerably by grafting polyalanyl side chains to gluten. Significant progress was made in relating solution properties of polypeptides to their structure. This research is being conducted by the Weizmann Institute of Science, Rehovot, Israel, under a PL 480 grant.

B. Industrial Chemical Products

1. Acid-modified flour. Acid-modified flour (AMF) and hydroxyethylated acid-modified flour (HEAMF), prepared in the pilot plant of the Northern Division, were used in machine trials at the Forest Products Laboratory. Running characteristics of both were acceptable although protein enrichment of recycled size solutions of both materials took place. Improvements in paper properties effected by both experimental materials were similar to those effected by the control, which was a high-grade, commercial hypochlorite-oxidized starch. Despite variations in the paper sized with the control, AMF appears to be a satisfactory product although this conclusion cannot be drawn categorically. Demonstration of essential equivalence of HEAMF and the control appears to be definite.

Laboratory studies on AMF indicated that unextractable protein (either bound to starch or denatured) is the most likely cause of selective uptake of carbohydrate and accompanying lack of penetration when AMF is used as

a paper size. Brightness of clay coatings made with AMF or hydroxyethylated AMF had previously been reported to be significantly higher than for coatings made with commercial starch products. Observations of stored samples showed that this superiority has persisted for a 2-year period. Rheological properties and adhesive strength of the coatings equaled or exceeded controls.

Engineering studies on the process for producing AMF showed that satisfactory products can be made by using gaseous HCl, flour dried to 10 percent moisture, and a reaction time of about 3 hours. This gas-phase process is kinetically equivalent to that using dry flour and 4N aqueous HCl, and the products of the two procedures have chemically similar characteristics. Reaction time can be shortened to less than 30 minutes by operating at 110°F. Cost estimates indicate a cost-to-make of about 2 cents per pound exclusive of flour cost for the 4N HCl process. For the gas-phase process at higher temperatures, cost-to-make is reduced to about 1.5 cents per pound. Calculations assumed a plant making 12 million pounds of AMF annually.

2. Flour xanthates and xanthides. In addition to starch xanthates and xanthides (see Area No. 1, Part B-1), the analogous derivatives of wheat flour, bran, ground wheat and related materials produce advantageous improvement in properties when incorporated into pulp and paper products. A mixer-kneader is being used successfully for continuous production of cereal xanthates in the absence of inert diluents. Studies to establish conditions for optimum results are still in progress; however, present data show that for degrees of substitution in the range of 0.07 to 0.17, 87 to 80 percent, respectively, of the added carbon disulfide reacts to form xanthate with a residence time in the mixer of 2 minutes. Data from all runs were submitted to Biometrical Services for statistical analysis to obtain information on the effects of conditions in production of xanthates with the mixer-kneader. A continuous 6.5-hour "production run" of xanthate was accomplished successfully.

Insulating wall boards were prepared containing flour xanthate crosslinked and insolubilized with zinc. Boards containing 10 percent of flour zinc xanthate were lighter but stronger than commercial boards. The best experimental boards were 1-1/2 to 2-1/2 times as strong as commercial boards of similar density. Drainage time in preparation of the boards was significantly shortened. Industry has expressed much interest in this development.

Other studies are providing much new information on the chemistry of cereal xanthates. Some of the more important results include: (1) Discovery of conditions for xanthation by a method requiring no solvent; (2) improved techniques for crosslinking with zinc ion; and (3) demonstration with model compounds that the xanthate-xanthide redox system is apparently reversible in the presence of certain solvents and inorganic salts.

3. New copolymers from wheat starch. In contract research at Stanford Research Institute, new techniques have been developed whereby starch graft copolymers such as the following can be obtained: starch with up to 40 percent add-on of methyl methacrylate, graft density up to 1 branch/65 AGU; starch with up to 25 percent add-on of styrene, graft density up to 1 branch/52 or 100 AGU at same add-on; starch with about 16 percent add-on of butyl acrylate or 17 percent add-on of methyl acrylate, graft density for both about 1 branch/25 AGU. It was shown further that oil-in-water emulsion systems can be used effectively for grafting of styrene to starch. Insoluble acrylonitrile graft copolymers could be solubilized by partial hydrolysis of the CN groups, showing that these copolymers are not crosslinked as previously supposed.

The new and improved techniques for preparing these graft copolymers make it possible to prepare a wider variety of products and thus greatly enhance the opportunities for developing commercially useful materials. Studies have been initiated to evaluate graft copolymers for various applications in paper, such as wet-end additives, sizes, adhesives and flocculating agents.

4. Evaluation of wheat flour products for applications in the pulp and paper industry. Research on chemically modified wheat flour and related products is supported by evaluation studies to determine the quality and performance of these products in applications in the pulp, paper and paper-board industry. During the reporting period tests were conducted, for example, with wheat flour xanthates and xanthides, acid-modified flour, and wheat starch graft copolymers. Results of the evaluation studies are reported in conjunction with the general discussion of research on the specific wheat flour product.

5. Adhesives from gluten and dialdehyde starch (DAS). In contract research at Iowa State University, information is being developed on optimum conditions for the preparation and use of the gluten-DAS adhesive with regard to both workability and performance as an adhesive for wood and to the economy of adhesive production. Calcium hydroxide was the best solvent of seven systems studied for solid mixtures of DAS and gluten. Best strength of adhesive bond was obtained from a mixture of 40 parts of gluten with 2 parts DAS--800-900 p.s.i. Studies of aging the reactants, the reaction products, and the adhesive joints have been initiated to provide optimization of larger-scale studies.

C. Processing Technology

1. Fine grinding and air classification of wheat flours. Investigation of fine-grinding and air-classification properties of five flours from varieties of Oklahoma HRW wheat grown at seven fertilizer levels showed that individual variety and climatic conditions affected protein shift during fractionation of the flours to a greater extent than did variations

in fertilizer level. Softer varieties of HRW wheat responded better than harder varieties. Flours from three Michigan SWW wheats and three Indiana SRW wheats were successfully separated into low-protein fractions (below 3 percent) and high-protein fractions (over 20 percent). New Gaines wheat, a Pacific Northwest SWW wheat, responded better to air classification than Omar club wheat but not quite as well as Brevor SWW wheat.

Fractions from Bison and Pawnee HRW wheats were prepared, making a total of five varieties now being evaluated at the Western Division in baking tests. These varieties and the corresponding protein shifts resulting from fractionation are Bison, 60 percent; Pawnee, 36 percent; Triumph, 59 percent; Wichita, 50 percent; and Comanche, 39 percent. (Protein shift is defined as the percent of total nitrogen shifted into the high-protein fraction plus that shifted out of the low-protein fractions.) Sufficient quantities of fractions from the Michigan and Indiana wheats are now on hand to permit both baking evaluations at the Western Division and physical and chemical studies at the Northern Division.

Varietal differences appear to be the most significant factor influencing response of HRW wheat to fine grinding and air classification. Present results are representative of what can be expected until or unless basic investigation of the binding of endosperm starch and protein reveals new information that can lead to conditioning treatments capable of enhancing protein shift. Although it does not appear possible on the basis of present knowledge to obtain so-called industrial starch fractions from hard wheats, materials that can now be produced by fractionating HRW wheat flour may have definite advantages in baking and in certain industrial applications.

2. Wheat conditioning. Protein release, estimated microscopically, was evaluated in terms of temperature and moisture level during conditioning. Release appeared to improve as temperature increased and moisture decreased, with maximum release (6 percent) for wheat conditioned to 8 percent moisture at 70°. Good progress is being made in developing techniques for preparing specimens from wheat kernels for electron microscopic examination. Ultra-thin sections (200-500 Å) of aleurone cell walls and contents and of starchy endosperm tissue have been successfully prepared and examined. Wheat endosperm showed particles of undetermined nature embedded in the protein, but no special adhering layer around starch granules or between cell wall and protein could be noticed. Radial and inner tangential cell walls of aleurone cells were seen to be traversed by protein strands (plasmodesmata) which are absent in the outer tangential walls. Results of studies of the effects of conditioning of wheat on protein release, together with availability of practical techniques for use of the electron microscope, provide a basis for continued hope that means can eventually be found for improving the response of hard wheat flours to air classification.

3. Enzymes in malted wheat. In basic research conducted under contract by Kansas State University it was shown that earliest formation of α -amylase occurs near the embryo. The adverse effect of post-harvest dormancy on

malting of wheat appears to be due to the state of the embryo as well as to the presence of inhibitory substances in the seedcoat and/or endosperm. Formation or release of α -amylase in the endosperm was found to be stimulated by gibberellic acid and also by materials apparently coming from the germinating embryo. The effects of gibberellic acid seem to involve oxidative activity, since SH-blocking agents inhibited activation. Metabolic activity of the aleurone layer was also stimulated by gibberellic acid and by material from the embryo. Growth of the embryo appeared to be stimulated in turn by material from the endosperm or other parts of the seed.

4. Microbiology of flour. Research has been initiated on examination of the microflora of wheat, flour and flour products. The ultimate objective of the work is the development of methods and processes to reduce viable microorganisms in wheat flour as it is produced in the mill.

5. Quantitative measurement of wheat conditioning variables. In studies under a PL 480 grant at the Cereals Research Station, Research Association of British Flour Millers, St. Albans, England, effect of deep freezing, both continuous and intermittent, at high kernel moisture (25 percent) was investigated as a means of increasing endosperm fissuring. The treatments increased flour yield up to 4 percent without loss of color grade in Manitoba (hard) wheat. Preliminary treatments of this type on soft white U.S. wheats gave similar results with possibly a slight increase in protein release. Deep freezing increased endosperm fissuring, which might be regarded as a step toward increased protein release. However, the fissures do not occur selectively along the starch protein interface, as might be hoped, but rather between individual endosperm cells. Micro-wave heat treatment of wheat in which the temper moisture was concentrated in the bran resulted in deterioration, rather than in improvement, of milling quality.

PUBLICATIONS AND PATENTS REPORTING RESULTS
OF USDA AND COOPERATIVE RESEARCH

Chemical Composition and Physical Properties

Berger, A., and Yaron, A. (The Weizmann Institute of Science, Rehovot, Israel). 1962. Multichain poly- α -amino acids. In "Polyamino Acids, Polypeptides, and Proteins," ed. M. A. Stahmann. Proc. Internl. Symp., Madison, Wisconsin, June 19-24, 1961, pp. 13-19.*

Dimler, R. J. 1962. New developments in the chemistry of wheat gluten. Proc. 2nd Pacific Northwest Wheat Util. Conf., Spokane, Washington, Oct. 30-31, 1961, pp. 53-60.

Jones, R. W., and Dimler, R. J. 1962. Electrophoretic composition of glutens from air-classified flours. Cereal Chem. 39(4), pp. 336-340.

*Research supported by PL 480 funds.

Sela, M., Lupu, N., Yaron, A., and Berger, A. (The Weizmann Institute of Science, Rehovot, Israel). 1962. Water-soluble polypeptidyl gliadins. *Biochim. Biophys. Acta* 62(3), pp. 594-596.*

Industrial Chemical Products

Anderson, R. A., and Griffin, E. L., Jr. 1962. Gluten and starch from wheat flour. An improved process. *Die Stärke* 14(6), pp. 210-212.

Lancaster, E. B., Pope, L. A., and Griffin, E. L., Jr. 1963. Preparation of acid-modified flour for tub sizing. *Cereal Chem.* 40(3), pp. 269-276.

Naffziger, T. R., Swanson, C. L., Hofreiter, B. T., Russell, C. R., and Rist, C. E. 1963. Crosslinked wheat flour xanthate-wood pulp blends for insulating board. Published in part in *American Paper Convention Daily*, Feb. 20, 1963, pp. 22-23.

Rankin, J. C., Russell, C. R., and Samalik, J. H., Sr. Jan. 15, 1963. Process for preparing improved sizing agents from cereal flours. U. S. Patent 3,073,724.

Russell, C. R., Buchanan, R. A., Rist, C. E., Hofreiter, B. T., and Ernst, A. J. 1962. Cereal pulps. I. Preparation and application of cross-linked cereal xanthates in paper products. *Tappi* 45(7), pp. 557-566.

Smith, H. E., and Russell, C. R. Oct. 9, 1962. Process for preparing polysaccharide sulfates. U. S. Patent 3,057,855.

Smith, H. E., Russell, C. R., and Rist, C. E. 1962. Preparation and properties of sulfated wheat flour. *Cereal Chem.* 39(4), pp. 273-281.

Smith, H. E., Russell, C. R., and Rist, C. E. 1963. Note on the preparation and use of poly-2-vinylpyridine-sulfur trioxide as a sulfating agent for starch. *Cereal Chem.* 40(3), pp. 282-285.

Processing Technology

Dimler, R. J., and Pfeifer, V. F. 1962. Fine grinding and air classification of wheat flour. *Proc. 1961 Wheat Util. Conf.*, sponsored by Washington Association of Wheat Growers in cooperation with Washington Agricultural Experiment Stations, Spokane, Oct. 30-31, 1961, pp. 39-46.

Fleming, J. R.,¹ Johnson, J. A.,¹ and Miller, B. S.² (¹Kansas State Univ., AES, Manhattan; ²USDA Crops Research Div., Manhattan, Kansas). 1962. Wheat malting. Effects of gibberellins and certain synergists on enzyme production during the malting of wheat. *J. Agr. Food Chem.* 10(4), pp. 304-308.

Griffin, E. L., Jr. 1962. More versatile wheat flours by air classification. *Proc. First Natl. Conf. Wheat Util. Research*, sponsored by National Association of Wheat Growers; Western and Northern Utilization Research and Development Divisions and other agencies of the U. S. Department of Agriculture; the Nebraska Wheat Commission, Wheat Growers, and State Experiment Station; Western Wheat Associates; and Great Plains Wheat, Inc., Lincoln, Neb., Oct. 29-31, 1962, pp. 109-120.

*Research supported by PL 480 funds.

Stringfellow, A. C., Peplinski, A. J., and Griffin, E. L., Jr. 1963. Fractionation of Gaines wheat flour from the Pacific Northwest. Northwest. Miller 268(13), pp. 34-36.

Stringfellow, A. C., Pfeifer, V. F., and Griffin, E. L., Jr. 1962. Air classification milling results for wheat and other cereal flours. Baker's Dig. 36(4), pp. 38-40, 42, 76.

General

Dimler, R. J. 1962. Gluten--the key to wheat's utility. Proc. First Natl. Conf. Wheat Util. Research, sponsored by National Association of Wheat Growers; Western and Northern Utilization Research and Development Divisions and other agencies of the U. S. Department of Agriculture; the Nebraska Wheat Commission, Wheat Growers, and State Experiment Station; Western Wheat Associates; and Great Plains Wheat, Inc., Lincoln, Neb., Oct. 29-31, 1962, pp. 88-95.

Dimler, R. J. 1963. Gluten--the key to wheat's utility. Baker's Dig. 37(1), pp. 52-57.

Miller, D. L., and Griffin, E. L., Jr. 1962. Bridging utilization engineering development to industrial usage. North. Util. Res. Devlpmt. Div., Agr. Res. Serv., U.S. Dept. Agr. ARS-71-20, 2⁴ pp.

Senti, F. R. 1962. Cereal products in papermaking. Proc. First Natl. Conf. Wheat Util. Research, sponsored by National Association of Wheat Growers; Western and Northern Utilization Research and Development Divisions and other agencies of the U.S. Dept. Agr.; the Nebraska Wheat Commission, Wheat Growers, and State Experiment Station; Western Wheat Associates; and Great Plains Wheat, Inc., Lincoln, Neb., Oct. 29-31, 1962, pp. 49-64.

Senti, F. R., and Kohler, G. O.¹ (Western Util. Res. Devlpmt. Div., Agr. Res. Serv., U.S. Dept. Agr., Albany, Calif.). 1962. Cereals. In "Yearbook of Agriculture 1962." U.S. Dept. Agr., pp. 460-465.

AREA NO. 3: CORN, SORGHUM, AND OTHER FEED GRAINS
IMPROVED INDUSTRIAL, FEED AND FOOD PRODUCTS

Problem. Abundant quantities of corn, sorghum, and other feed grains are now available beyond those amounts required to satisfy current needs. Both domestic consumption and export potential of these grains could be increased by development of new products for use by industry or of improved foods and feeds. Industrially, increased use of corn and sorghum will be mainly dependent upon increased markets for starch. However, flours derived from these grains are mixtures of starch, protein, and minor amounts of other components. Such mixtures have promise as raw materials for conversion to adhesives, water-soluble coatings, plastic materials, and related products that should have properties and uses different from related products derived from refined starch or wheat flour and that should contribute independently to increasing industrial markets. Isolated protein components of corn and sorghum flours should be suitable raw materials for production of useful resins and films. To achieve these utilization goals, more information is needed on basic physical and chemical properties and reactions of these flours, on the properties of component lipids, waxes, and proteins and their possible interactions with starch, and on the use of fine grinding and air classification and other new milling techniques for obtaining milled products having the most advantageous properties as industrial raw materials.

Because of the growing emphasis on increasing meat production, there is need for processes to obtain improved feed products such as high-protein feeds, mill feeds, feed concentrates, and feeds with high oil content. Such improvement could be achieved through research to obtain better knowledge of the biologically and nutritionally important constituents of corn, sorghum, and oats, to evaluate present, and to develop improved, milling and processing methods, and to ascertain the effects of such methods on the nutritional qualities of the products. In addition, because of the world shortage of protein in human nutrition, this research could enhance the export value of these grains by providing the necessary basis for development of high-protein and other food products that would be acceptable in foreign markets.

USDA PROGRAM

The Department has a continuing long-term program involving analytical and organic chemists, chemical engineers and structural biologists engaged in basic studies of the components of corn and sorghum and in application of the new knowledge gained to the development of improved processing technology leading to more effective utilization of these cereal grains.

The Federal scientific effort for research in this area totals 6.7 professional man-years. Of this number 4.4 are devoted to chemical composition and physical properties and 2.3 to processing technology.

Research on chemical composition and physical properties is conducted at Peoria, Illinois, and involves investigations of physiologically active nonprotein nitrogen substances in corn and of carotenoid pigments of corn, corn milling fractions, and yellow endosperm sorghum. A portion of the effort on carotenoid pigments is cooperative with Crops Research Division and is directed to development of corn and sorghum varieties having high carotenoid content. Such varieties are needed for improved food and feed products and to enhance the competitive position of U. S. corn in international trade.

Processing technology research, also conducted at Peoria, Illinois, involves pilot-plant studies of conditions and methods for increasing the yield of oil and grits by dry-milling processes. Effects of processing variations on industrially and biologically important components of corn are determined. During the reporting period research on dry milling of corn was redirected to emphasize studies on tempering, on improved processing of old or artificially dried corn, and on development of improved degermination equipment.

The Department also sponsors research in this area conducted under grants of PL 480 funds to the following foreign institutions: Research Association of British Flour Millers, St. Albans, England, for studies of antioxidants occurring in oats (5 years, 1960-1965); National Institute of Agronomic Research, Paris, France, for basic studies of the physical chemical properties of corn zein (4 years, 1961-1965); Weizmann Institute of Science, Rehovot, Israel, for research on synthetic polypeptides with amino acid compositions related to zein and wheat gliadin (4 years, 1960-1964); and Indian Institute of Science, Bangalore, India, for research on separation of grain sorghum proteins (5 years, 1963-1968). These lines of work are under the subheading chemical composition and physical properties.

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition and Physical Properties

1. Nonprotein nitrogen substances of corn. New basic information has been obtained on the composition of corn and corn steep liquor. Quantitative estimation of heterocyclic nitrogen bases in corn steep liquor showed high levels of cytidine, uridine, xanthine, guanosine and adenine. Certain of these substances were not present in the original corn and others were present in steep liquor in larger amounts than anticipated from the composition of the original corn, indicating enzymatic degradation of nucleic acids and modification of bases during steeping.

Four previously unidentified amino acids found in corn extracts were characterized as homoserine, β -alanine, α -aminobutyric acid and α -amino-adipic acid. Comparison of different batches of corn steep liquor revealed significant differences in amino acid composition, especially arginine and ornithine. This type of information is potentially important to the processing and utilization of corn and derived products. For example, the kind and concentration of heterocyclic nitrogen bases may explain the stimulating effect of corn steep liquor on growth of microorganisms. Another possibility is that certain of these bases may be related to so-called unidentified growth factors said to occur in corn steep liquor.

2. Corn and sorghum carotenoids. Work was continued on investigations of carotenoid pigments in corn and sorghum to provide basic information needed in studies of the effects of processing on these biologically important constituents. Comparison of carotenoids in "normal-yellow" and "lemon-yellow" types of corn showed that the normal corn contained four times as much carotenoid pigment as did the other type and that α -carotene, β -carotene, zeinoxanthin and cryptoxanthin were present only in the normal corn. However, the lemon-yellow type contained five times as much zeta-carotene as did normal corn.

In cooperative research with Crops Research Division designed to assist in development of new varieties of corn and sorghum containing increased amounts of carotenoid pigments, some 450 hybrid corn samples were analyzed for xanthophylls and carotenes. Indications were obtained of the presence of gene modifiers in the white parent of yellow by white crosses.

Carotenoid analyses of nine new yellow-endosperm sorghum grains (protected from weathering) gave a range in carotenes from 0.3 to 1.6 ppm and xanthophylls from 2.7 to 10.0 ppm. These differences in the carotenoid levels of the yellow-endosperm sorghum grain samples are significant and indicate that several of the strains should be considered in future breeding programs.

3. Synthetic polypeptides related to corn zein protein. Good progress is being made in relating solution properties of polypeptides to their structure. For example, optical rotatory dispersion properties of helical polypeptides indicate that their unfolding by acids and bases is due to repulsion of electrically charged groups in side chains rather than to any effects of hydroxyl or hydrogen ions on hydrogen bonds in the α -helix. Synthetic work pertained to the wheat gliadin phase of this research, which is being conducted under a PL 480 grant at the Weizmann Institute of Science, Rehovot, Israel.

4. Properties of corn zein protein. At the National Institute of Agronomic Research, Paris, France, initial studies on commercial zein using column fractionation, rotatory dispersion, determination of molecular shape and weight by ultracentrifugation and light scattering, and amino acid composition have provided a foundation of techniques and information needed for

continuation of the research. The commercial zein showed a weight average molecular weight of about 35,000, and evidence was obtained that the molecule is relatively elongated. A new approach to protein fractionation was initiated using a column procedure with a gradient of both solvents and temperature. This work is being conducted under a PL 480 grant.

B. Processing Technology

1. Corn dry milling. Studies on milling of old corn showed that time and moisture level of tempering strongly influenced degerminator throughput and characteristics of the products. Best overall results were obtained with a 2-hour temper at moisture levels of 18-24 percent. Hot tempering decreased degerminator throughput. Other studies showed that for both 1960 and 1961 crop corns, vacuum tempering gave a higher throughput than did conventional tempering of equal time (2 hours). With a 0.4-hour vacuum temper a slightly better oil recovery was achieved. The improved hull release obtained with vacuum tempering of corn may reduce or eliminate the need for a second temper, thereby increasing the efficiency of commercial operations. In processing old corn, mills experience difficulty in obtaining adequate tempering and germ recovery. Vacuum tempering may prove advantageous in this operation.

A prototype brush-impact corn machine (designed and built at the Northern Division), when run as a dehuller, gave essentially whole dehulled kernels (12 percent had attached hulls) with no germ release. When the machine was run as a dehuller-degerminator the product was comparable to that produced by the Beall degerminator except that the grits had a higher oil content. Sorghum gave very good germ and hull release in the experimental degerminator. Horny and floury endosperm flours from six sorghum varieties resembled hard wheat flours in response to air classification. These results with corn and sorghum appear to point the way to significant improvement in dry milling technology.

PUBLICATIONS AND PATENTS REPORTING RESULTS OF USDA AND COOPERATIVE RESEARCH

Chemical Composition and Physical Properties

Blessin, C. W., Dimler, R. J., and Webster, O. J.¹ (¹USDA Crops Res. Div., Lincoln, Neb.). 1962. Carotenoids of corn and sorghum. II. Carotenoid loss in yellow-endosperm sorghum grain during weathering. Cereal Chem. 39(5), pp. 389-392.

Blessin, C. W., VanEtten, C. H., and Dimler, R. J. 1963. An examination of anthocyanogens in grain sorghums. Cereal Chem. 40(3), pp. 241-250.

Christianson, D. D., Wall, J. S., Cavins, J. F., and Dimler, R. J. 1963. Chromatography of quaternary nitrogen compounds on buffered cation-exchange resins. J. Chromatography 10(4), pp. 432-438.

Wall, J. S., Dimler, R. J., and Senti, F. R. 1962. New information on composition and nutritive properties of corn. Proc. Symp. What's New in Production, Composition, and Utilization of Corn, sponsored by Midwest Section, Am. Assoc. Cereal Chem., Jan. 10, 1961, Chicago, Ill., pp. C-1-C-11.

Processing Technology

Brekke, O. L., Weinecke, L. A., and Griffin, E. L., Jr. 1962. Corn dry-milling: Influence of feed rate and tail-gate loading upon Beall degerminator performance. Cereal Chem. 39(5), pp. 381-388.

Weinecke, L. A., Brekke, O. L., and Griffin, E. L., Jr. 1963. Vacuum-tempering corn for dry-milling. Cereal Chem. 40(2), pp. 187-193.

AREA NO. 4: HIGH-AMYLOSE CORN
INDUSTRIAL UTILIZATION

Problem. Varieties of corn have been achieved genetically that contain greatly increased amounts of amylose. Amylose, the linear fraction of starch, possesses film- and fiber-forming properties not available in ordinary starch which contains only about 27 percent of this component. Because the unique properties of amylose open areas of utilization closed to ordinary starch, the potential industrial value of this new crop is very high. Several problems must be solved, however, to realize this potential.

For high-amylose starch to have substantially improved properties as a raw material in comparison with ordinary starch, it should contain at least 80 percent of amylose. A few breeding samples have recently been observed that contain over 80 percent of amylose. However, only varieties containing 50 up to about 70 percent have so far been commercially available. Even at this amylose level, however, over 4 million pounds of high-amylose starch from first commercial plantings were utilized by industry in 1961. Although breeding is the task of the geneticist, utilization research is needed to provide information on amylose content, on changes in quantities and properties of the amylose, amylopectin, and other components such as oil and protein, and on milling characteristics of breeding samples in order to insure availability of satisfactory varieties.

A second problem is development of methods for economical isolation of pure amylose from high-amylose starch. For some anticipated uses, nearly pure amylose may be required for optimum properties. Although achievement of 80-percent high-amylose starch appears certain, it may not be possible to obtain much higher levels through breeding. Success in devising efficient fractionation methods will depend upon availability of adequate basic information on freeing and separating amylose from remaining starch components.

Finally, to insure utilization of the potentially large volume of high-amylose starch that could eventually become available, more information is needed on the chemical and physical properties of amylose and high-amylose starch and on methods for converting them economically to desired products. Success in this research could lead to an estimated consumption of over 600 million pounds of high-amylose starch by 1975 in films, fibers, plastics, coatings, and related products to which the linear character of amylose could make contributions.

USDA PROGRAM

The Department conducts a long-term, continuing program of research involving analytical, organic and physical chemists, structural biologists, and chemical and mechanical engineers who are engaged in basic and applied

research designed to increase knowledge of the properties and reactions of amylose and other components of high-amyllose corn and to utilize this knowledge in development of attractive industrial applications for amylose and high-amyllose starch.

The Federal scientific effort for research on utilization of high-amyllose corn totals 19.3 professional man-years. Of this number 14.7 are devoted to chemical composition and physical properties and 4.6 to industrial utilization.

Research at Peoria, Illinois, on chemical composition and physical properties (14.5 professional man-years) involves study of amylose content of breeding samples, starch and starch granule composition, structure and properties; and composition and properties of proteins and other components of high-amyllose corn. Studies on amylose content of breeding samples assist geneticists in developing varieties of high-amyllose corn having increased amylose content. Cooperation with Field Crops Research Branch, Crops Research Division, is maintained in conducting these studies. A research contract (0.2 professional man-year) is in effect at Arizona State University, Tempe, Arizona, for basic research on the interaction of "V" amylose with small organic molecules. Research on industrial utilization, which is conducted at Peoria, Illinois, is devoted to studies on separation of amylose from high-amyllose starch, and formation and properties of amylose films (4.6 professional man-years). Initial phases of research on amylose films were completed during the reporting period.

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition and Physical Properties

1. Amylose content of breeding samples. During the reporting period 15,867 samples of high-amyllose corn were analyzed. These were supplied under Memoranda of Understanding by the Bear Hybrid Corn Company, by the Missouri Agricultural Experiment Station and by Crops Research Division at the Missouri Station. Of the samples analyzed in the last 6 months of the period, nearly 12 percent contained 75-80 percent amylose, thus increasing the number in this range from the previous total of 250 to a total of 1,132 to date. In addition, a total of 10 samples containing over 80 percent of amylose have now been found. The highest value was 81.3 percent. (In this report percentages of amylose refer to apparent values determined by iodine titration. True amylose content, measured by quantitative fractionation, averages about 80 percent of the apparent value.) These results indicate that the development of high-amyllose corn is proceeding to the objective in a satisfactory manner. Reports indicate that moderate quantities of high-amyllose starch containing 70 percent of amylose are now available commercially.

2. Properties of components of high-amylase starch. Studies on structure of amylose and amylopectin (from 52-70 percent high-amylase corn) show a number average chain length for amylose of 490 AGU. (The term AGU refers to the glucose units of which starch is composed.) Average branch length of amylopectin was 36-42 compared to 27 for that from ordinary corn starch. For the high-amylase corn amylopectin the length of the inner segment was about the same as that for ordinary amylopectin but the chain length of the external branches increased with increase of amylose in the starch. Improvements in techniques for separation and molecular weight determination gave increased values compared to those previously reported. Present results for weight average molecular weights are 470,000 for dent corn amylose and 380,000 for amylose from 70-percent high-amylase corn.

More complete studies on neutral solvent systems revealed that a mixture of lithium thiocyanate and guanidinium thiocyanate will completely dissolve high-amylase starch at room temperature. Discovery of effective neutral solvents for starch is an important advance because use of these solvents minimizes chemical changes in starch and thus permits more accurate determination of molecular weights and other significant properties.

Whereas iodine sorption indicates 25-27 percent of amylose in dent corn, ultracentrifugal schlieren patterns indicated 32-33 percent in the molecular weight range of amylose. The higher value was shown to be due to the presence of a low-molecular-weight (400,000) material having a degree of branching of 4.1 percent, a value similar to that of normal dent corn amylopectin. Discovery of a low-molecular-weight amylopectin-like component in starch is important from both the theoretical and practical points of view. Further study and characterization of this "anomalous component" in dent, high-amylase and other corns should contribute to our knowledge of the formation of starch and starch granules and increase our understanding of the chemical and physical properties of corn starches.

3. Proteins of high-amylase corn. Examination by starch gel electrophoresis of various protein fractions of waxy, normal dent and amyloplasmid hybrid corns (isogenic except for wx and ae genes in the waxy and amylose types) revealed no differences in number or relative amounts of components detected in the zein, globulin and glutelin fractions. Gel electrophoretic studies of reduced zein, globulin and glutelin preparations from normal dent corn showed that components in the reduced glutelin had counterparts in either the reduced zein or reduced globulin preparations. No components unique to the glutelin preparation were observed.

Gel filtration on Sephadex removed pigments from corn globulins but did not resolve individual globulin components. Chromatography on carboxymethyl- and DEAE-cellulose yielded fractions of much less complexity but again did not resolve individual components. An apparently homogeneous component was, however, obtained from the globulin fraction by a salt-precipitation technique.

These studies are providing basic information on corn proteins that will be applicable to the investigation and interpretation of variations in processing characteristics and to the detection of alterations of constituents by processing.

B. Industrial Utilization

1. Fractionation of high-amylose starch. In engineering research, a technically feasible process has been developed for recovery of amylose from high-amylose starch by complexing with selected fatty acids and alcohols. Application to commercial high-amylose starch (60 percent apparent amylose) gave good yields of amylose of 85- to 90-percent purity. Good separations could not be obtained using the commercial procedures presently employed for dent corn starch.
2. Amylose films. By extrusion of amylose dispersions at -16 to -19° C. into the eutectic mixture of ammonium sulfate, sodium sulfate and water, unsupported amylose film has been produced at speeds up to 400 feet per minute. The film loses strength, however, during subsequent washing prior to drying by conventional means. Use of nonaqueous media for washing may prove to be advantageous. For example, dehydrating wet, unwashed coagulated amylose film in glycerol gave a film having a wet tensile strength of 1,000 lbs. per sq. in. Evaluation of hand-cast films prepared by the alkali-dispersion method from a variety of amylose materials indicated that a minimum of 75 percent amylose is necessary for good film production; that amylose content was, within limits, more important than intrinsic viscosity; and that defatting was necessary to produce films with good forming, handling, and final properties.

PUBLICATIONS AND PATENTS REPORTING RESULTS OF USDA AND COOPERATIVE RESEARCH

Chemical Composition and Physical Properties

Anderson, R. A., Uhl, D. E., Deatherage, W. L., and Griffin, E. L., Jr. 1962. Composition of the component parts of two hybrid high-amylose corns. *Cereal Chem.* 39(4), pp. 282-286.

Anderson, R. A., and Uhl, D. E. 1963. Modification of the polarimetric starch determination on high-amylose corn. *Cereal Chem.* 40(1), pp. 87-88.

Montgomery, E. M., and Sexson, K. R. July 24, 1962. Recovery of amylose from high amylose cornstarch. U. S. Patent 3,046,161.

Processing Technology

Anderson, R. A. 1962. A note on the wet-milling of high-amylose corn containing 75-percent-amylose starch. *Cereal Chem.* 39(5), pp. 406-408.

AREA NO. 5: WHEAT AND CORN
FERMENTATIVE CONVERSION TO NEW INDUSTRIAL, FEED AND FOOD PRODUCTS

Problem. By fermentation of cereal grain substrates, new products can be obtained that are not readily available by other means and have promising potential for industrial, agricultural, and food uses. Processes now under development, if brought to successful conclusion, could lead to increased consumption of an estimated 40 million bushels of grain for fermentative conversion to stable viscosity agents for secondary petroleum recovery by flooding of spent oil wells, to new organic acids and enzymes for industrial use, to feed supplements, and to effective biological insecticides and other pesticides that are harmless to man. In addition, there are good possibilities for utilizing fermentation processes to produce new food products that should promote foreign use of U. S. grains.

To accomplish these objectives and to realize the full potential of fermentative techniques for increasing utilization of grain, a broad program of exploratory research is required to find and identify through taxonomic studies species of organisms producing potentially valuable products, to isolate high-yielding strains or develop them by mutation, hybridization or genetic selection, and to develop basic information on culture media, special nutrients, and other factors required for optimum growth of microorganisms and maximum yields of desired products. Continued maintenance and expansion of a collection of pure cultures of well-characterized organisms is necessary for this research. For successful translation of laboratory results into commercially useful processes, more information is needed on new techniques of fermentation, on development of economical methods of growing organisms and handling fermentation processes on a large scale, and on special procedures for efficient isolation and purification of products from fermentative reaction mixtures. Finally, the most appropriate end uses for products must be identified and information obtained on product evaluation and development.

USDA PROGRAM

The Department has a long-range continuing program involving analytical and organic chemists, biochemists, microbiologists, systematic biologists, and chemical engineers engaged in basic research on microorganisms and microbiological reactions and products and in application of both known and newly discovered principles to the development of practical fermentation processes for conversion of cereal grain substrates to useful chemical, biological, feed and food products.

The Federal scientific effort in this area of research totals 55.3 professional man-years. Of this number 13.8 are devoted to basic research on fermentation processes; 21.3 to industrial chemicals; 15.3 to biological pesticides; and 4.9 to feed and food products.

Basic research on fermentation processes conducted at Peoria, Illinois, (13.8 professional man-years) includes study of taxonomy of molds, yeasts and bacteria; factors affecting viability of microorganisms; and microbiological reactions and products. Basic to these investigations and to the Division's entire research program on fermentation is assembly and maintenance in pure culture of a large collection of agriculturally and industrially important microorganisms. Much of the research on microbiological reactions and products is conducted by the Pioneering Laboratory for Microbiological Chemistry. During the reporting period exploratory research on microbial amination of unsaturated fatty acids was discontinued. Research on taxonomy of Pseudomonas is being held in abeyance to permit assignment of personnel to more urgent problems.

Research at Peoria, Illinois, on industrial chemicals (21.0 professional man-years) involves fermentative production of microbial gums, organic acids, and other products for use in the chemical industry. This work includes investigation and development of improved or new procedures for conducting industrial fermentations. A research contract (.3 professional man-year) with the University of Arizona provides for studies on polymerization of selected fermentation acids and derivatives of fatty acids.

Research at Peoria, Illinois, on biological pesticides (12.5 professional man-years) is devoted to studies on biological insecticides for Japanese beetle, other insect control agents and plant antibiotics. Investigations on biological insecticides for Japanese beetle and on other insect control agents is cooperative with Entomology Research Division and Plant Pest Control Division. Research on plant antibiotics involves cooperation with Crops Research Division. During the reporting period research on insect attractants was completed. Research contracts (2.8 professional man-years) covering various phases of research on Japanese beetle pathogens are in effect at Michigan State University, East Lansing, Michigan, for study of factors important to large-scale propagation of the pathogens (.5 professional man-year) and for basic research on enzyme activity in sporulation (.7 professional man-year); at Kansas State University, Manhattan, Kansas, for investigation of stabilization of vegetative cells of the pathogenic organisms (.5 professional man-year); at the University of Minnesota, St. Paul, Minnesota, for fundamental studies on the transfer of genetic determinants of sporulation from one microorganism to another (.5 professional man-year); and at the University of Illinois, Urbana, Illinois, for research on the applicability of a sporulation factor produced by bacteria to Japanese beetle pathogens (.6 professional man-year).

Research at Peoria, Illinois, on feed and food products (4.4 professional man-years) involves study of production of microbial carotenoids suitable for feed supplements and development of new fermented wheat foods that can help increase export markets for U. S. wheat. A research contract (.5 professional man-year) with Michigan State University, East Lansing, Michigan, concerns evaluation of biological availability of fermentative β -carotene when fed to poultry and swine.

The Department also sponsors research in the fermentation area conducted by foreign institutions under grants of PL 480 funds. Basic research on fermentation processes involves grants to the National Institute for Agronomic Research, Madrid, Spain, for collection of new species of yeast (5 years, 1960-1965); University of Helsinki, Finland, for basic studies on organic phosphorus compounds of yeast (5 years, 1960-1965); University of Milan, Italy, for basic studies on the metabolic pathway to 2-ketogluconic acid in Acetobacter species (4 years, 1960-1964); University of Allahabad, India, for collection of new Mucorales species (5 years, 1961-1965) and studies on survival of lyophilized microorganisms (5 years, 1962-1967); University of Durham, Newcastle-upon-Tyne, England, for investigations of sugar phosphate derivatives in molds (5 years, 1962-1967); and Indian Institute of Science, Bangalore, India, for basic research on enzyme systems involved in Pseudomonas conversion of glucose (5 years, 1962-1967). Research on industrial chemicals involves a grant to Superior Institute of Health, Rome, Italy, for studies on foaming in anaerobic fermentations (2 years, 1961-1963). Research on feed and food products involves a grant to the "Giuliana Ronzoni" Scientific Institute of Chemistry and Biochemistry, Milan, Italy, for research on production of vitamin B₁₃ (5 years, 1960-1965).

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. Basic Research on Fermentation Processes

1. ARS Culture Collection maintenance and service. As of January 1, 1963, the ARS Culture Collection contained 13,559 permanent cultures, an increase of 3,576 over 1962. During calendar year 1962, 2,497 cultures were distributed, 1,852 to domestic addresses and 645 to foreign countries.

In a recent search of the records, the Culture Collection reservoir was found to contain representatives of only 8 genera of Tuberculariaceae out of approximately 150 known genera in this family of Fungi Imperfecti. Most of the isolates belong in 3 genera, namely Fusarium, Epicoccum, and Myrothecium. Since this observation, 17 strains of 8 genera, including representatives of 4 genera new to the Collection, have been added. Of these, 3 isolates from fescue seemingly represent a new species of Lomachashaka, a monospecific genus reported only once in the literature. The monospecific genus Volutina has been reported only twice and the fungus was not obtained in pure culture in either case. We now have 2 cultures, one that we isolated and another that was sent to us by Wayne State University, Detroit. These presumably are the only pure cultures of these organisms in existence.

Recently, 8 isolates of Aspergillus flavus were received from the Commonwealth Mycological Institute (England). These strains have experimentally produced substances toxic to ducklings. They were isolated in England at the Central Veterinary Laboratories from peanuts and peanut meal from Nigeria, Uganda, and Brazil.

At the University of Allahabad, India, a large number of interesting and different forms of Mucorales have been isolated. These represent 52 different species including some new ones. All new and significant cultures are sent promptly for incorporation into the ARS Culture Collection. In studies on survival of lyophilized cultures, also in progress at the University of Allahabad, over 100 cultures of Aspergillus niger, Aspergillus terreus, etc., have been isolated. A number of morphological and biochemical characteristics are being determined for study with respect to alterations during repeated lyophilizations. These investigations are being performed under PL 480 grants.

2. Bacteria investigations. Emphasis was placed on antibiotic production by strains of one taxonomic group of Actinomycetes (produces streptomycin, rhodomycin, cycloheximide, etc.). Antibiotic spectra, cross-antagonism activities, and paper-chromatography patterns (using three solvent systems and five test organisms) afforded further information that can be used to determine how precisely cultural characteristics and antibiotic-producing capacity (qualitative) are related. This knowledge, when combined with the usual morphological data, will allow more objective characterization of strains and should aid in correcting current misinformation on taxonomy of streptomycetes.

Research on taxonomy of Pseudomonas is being held in abeyance to permit assignment of personnel to the Japanese beetle milky disease problem.

3. Yeast investigations. Studies on Chlamydozymaceae, a new family of yeasts, have resulted in discovery of a new type of sexuality and inheritance mechanism of extremely primitive character. A new species of Chlamydozyma was found to have hybridizing characteristics that will facilitate correct designation of the sexes of these yeasts.

A yeast responsible for a very persistent infection of cadavers that were injected with embalming fluid and stored in 4-percent phenol solution was sent to the Northern Division by a medical school for identification. The infection had resisted all available microbicides and was terminated only when the medical school occupied completely new quarters with different cadavers, new storage tanks and uncontaminated preservatives. The yeast was found to be a new species of Hansenula and to exist in two readily interchangeable types, one oxidative and one fermentative. The yeast has been named H. petersonii. A new theory according to which mutations regulate the most economical synthesis of enzymes for utilization of sugars or other metabolites is proposed on the basis of the fermentative-oxidative shift in H. petersonii.

4. Mold investigations. A cup fungus, Plectania occidentalis, has been found to produce on a glucose substrate yields as high as 35 percent of a new polysaccharide consisting of at least 90 percent of glucose units. No other sugar has been found, but traces of uronic acid have occasionally

been indicated. The same polymer is produced from either glucose or xylose as a substrate, but although starch, maltose and sucrose appear to be utilized, no polymer is formed with these substrates. Viscosity of solutions is not significantly affected by salts except borates nor by acid or alkali at moderate temperatures. Polymer solutions have been autoclaved for 2 hours in the presence of calcium chloride without change. The polymer is comparatively inert to microbial attack.

Production of a polysaccharide by a cup fungus is a phenomenon of unusual interest since it is believed that heretofore no product except cells was known to result from gross carbon turnover by a higher fungus. Both the fermentation and the polymer have characteristics indicating potential industrial value. In addition, this discovery raises several questions of fundamental scientific importance. For example, what is the mechanism whereby either the 6-carbon sugar glucose or the 5-carbon sugar xylose are converted to a glucose polymer but no polymer is produced from maltose, starch or sucrose even though these carbohydrates yield glucose on hydrolysis? What structural features of the polymer account for its properties in comparison with other fermentation polysaccharides? Answers to such questions would be significant contributions to basic science as well as provide knowledge that could be applied in our research program in several areas.

Taxonomic research on Mucorales revealed three varieties (two of which are new) of Rhopalomyces elegans, the microorganism that parasitizes eggs of the nematode genus Rhabditis. A new species of Absidia, named A. californica, was discovered. A surprising number of new taxa are being found in the several hundred isolates of Absidia examined. The sexual stage has been found in every species so far studied. More complete taxonomic information was attained on Absidia ramosa-corymbifera. This is undoubtedly the most common and economically important species of Absidia. It occurs in koji, stored grains, heating hay, curing tobacco and sewage. Its study completes work scheduled for the genus Absidia.

5. Microbiological processes and products. In the Pioneering Laboratory for Microbiological Chemistry, studies on the formation of D-arabitol by Saccharomyces mellis showed that the reduction in sugar alcohol production observed when phosphate is in the medium was caused by repression of the enzyme responsible for removal of phosphate from the intermediary product, D-ribulose-5-phosphate. Other steps in the conversion of glucose to D-arabitol were also elucidated. Evidence has been obtained that isolated cell particles from photosynthetic bacteria must undergo some physical change before becoming photochemically active in electron transport reactions. An active hydrogenase unaffected by the presence of light or calcium ion was shown to be present in cell-free extracts of Rhodospirillum rubrum.

Research on macromolecules responsible for agglutination of opposite mating types of the yeast Hansenula wingei resulted in isolation of a soluble factor from type 5 that has properties similar to living type 5 cells. Thus, the factor agglutinates cells of the opposite mating type 21, it is adsorbed from solution only by active mating type 21, and, like the parent type 5 cells, it is inactivated by agents that break disulfide bonds. This factor is therefore one, if not the only, specific agglutinating factor present on the surface of type 5 cells. Purification and characterization of the factor are in progress.

Research is continuing on the characterization of an extracellular lipid produced by H. ciferrii, on new aspects of fonsecin structure revealed by nuclear magnetic resonance, and on the metal-chelating properties of ramulosin. In the course of these chemical studies a new reagent, dicyclohexylmethylethylamine, was found to give superior results in the small-scale formation of phenacyl and methyl esters of carboxylic acid. These esters are important derivatives that are widely used in characterization of organic compounds.

In research under a PL 480 grant at the University of Milan, Italy, stable cell-free preparations of Acetobacter have been developed that have activity in causing specific conversions expected to be part of the pathway from glucose to 5-ketogluconate. Of particular significance is the ability of the particulate fractions of cells to form 5-ketogluconate. The work should ultimately define the pathway and thereby provide a possible basis for control of fermentations done with whole cells.

At the University of Helsinki, Finland, the major nucleotide and sugar phosphate components of Torulopsis utilis were identified and separated by a rapid two dimensional paper chromatographic method. A short-term radioactive tracer labeling technique was developed, and preliminary metabolic turnover experiments were conducted. These techniques are being applied to the study of carbohydrate metabolism in yeast under various physiological conditions. This work is being performed under a PL 480 grant.

Studies on microbial sugars, their phosphate derivatives, and related compounds, which are conducted under a PL 480 grant by the University of Durham, Newcastle-upon-Tyne, England, have revealed that the polymers of Penicillium griseofulvum contain mannose, glucose and galactose as do other Penicillium organisms and that they also contain phosphates. Two of the polymers of Streptomyces niveus had a repeating unit of glucose and mannose, whereas a third had glucose, glucosamine and phosphate. The nucleotide fraction of the streptomycete is being studied in order to provide a better understanding of the biosynthesis of antibiotics and polysaccharides.

B. Industrial Chemicals

1. Conversion of grains to fermentation media. A laboratory process has been developed for enzymatic conversion of the starch in ground grains to glucose-containing sirups that are economical media for production of industrial chemicals and antibiotics by fermentation. The method, which is based on α -amylase and amyloglucosidase, is effective for ground sorghum, ground corn, corn flour and corn meal. Yields of glucose from sorghum and corn amount to 95 percent of theoretical. Solids remaining after enzymolysis of sorghum amounted to 23 percent and contained 4 percent nitrogen. About one-fifth of the nitrogen in the original sorghum was solubilized. For corn the residue was 26 percent and contained 3 percent nitrogen. About one-third of the nitrogen in the original corn was solubilized. Preliminary calculations indicate that on a sugar basis cost of the sirup from corn is probably less than that of molasses products. Enzymatic conversion of ground corn and sorghum and related materials to glucose sirups has elicited much interest from industry as evidenced by the large number of inquiries by letter, telephone, and personal visits by industrial representatives.

2. Enzymatic modification of wheat flour. Conditions for a two-step enzymatic conversion of wheat flour to a preparation that could be used as a surface size or coating for paper have been worked out using pepsin and bacterial subtilisin as the proteolytic enzymes and bacterial α -amylase as the amylolytic enzyme. The gluten can be degraded to either large or small molecular-weight fragments depending on the choice of proteolytic enzyme. Study of flour fractions containing 2 to 24 percent protein showed that those containing 2 to 5 percent required large amounts of amylase and long reaction times. The higher protein fractions required less amylase and time but yielded sizing material of lower quality. By treating a fraction containing 7 percent protein with amylase only, a product was obtained that compared favorably (as a tub size) with Super-film 40 (a superior commercial sizing starch). Production of an enzymatically converted flour fraction comparable, as a tub size for paper, to a leading commercial starch product is encouraging. The contrasting results with high and low protein flour fractions point up the necessity for evaluating the effects of enzymes originally present in the flour.

3. Fermentation acids. Engineering studies on fermentation of wheat starch slurry (from the batter process for production of wheat gluten) to citric acid, were continued with emphasis on scaling-up the process. As previously reported, the microorganism used for the fermentation consumes 75 to 80 percent of the starch of the medium. A similar result was obtained with a synthetic medium containing 10 percent glucose. Complete sugar utilization was achieved by addition of enough calcium carbonate to neutralize one-half of the anticipated citric acid yield. Yields of citric acid amounting to 65 percent by weight (based on glucose) have been achieved in 60-liter fermentors.

Specifications of the research contract with the University of Arizona provide for study of vinyl derivatives of fatty as well as fermentation acids. Under this contract, which is sponsored by all four utilization research laboratories, work related to the Northern Division commodities has so far dealt with vinyl derivatives of fatty acids. Studies on use of such derivatives as internal plasticizers for poly(vinyl chloride) show that both the plasticizing ability of the internal plasticizer as well as its influence on side-chain crystallinity are factors of major importance.

4. Studies on continuous fermentation techniques. Since the last report, one producer of B-1459 microbial polysaccharide has installed a commercial-size fermentor and is considering installation of two more. One company has reportedly made a 50,000-gallon run. There is active interest in the QAC (quaternary base) method for isolating the product and at least two companies are evaluating the economics of the process. At this critical stage in industrial development of B-1459 polymer, the importance of finding the most economical conditions for production cannot be overemphasized. The problem is being attacked by initiation of research on the use of continuous fermentation techniques and on simplification of the fermentation medium.

In the pilot plant, equipment has been modified for continuous fermentation and some preliminary testing has been completed. Laboratory studies on simplification of the medium showed that either soybean meal or "Fermatein" (a commercial cottonseed meal preparation) gave equal or better yields than distillers' solubles. Ash content of the product was reduced by lowering the concentration in the medium of phosphate buffer and magnesium sulfate. Soybean meal and Fermatein are cheaper than distillers' solids and indications are that only 40 to 50 percent as much of these less expensive materials would be required. However, further work is required to establish that their use does not change properties of the polymer or interfere with its recovery. For both conventional and continuous fermentation, a medium having all components in solution would be highly desirable.

5. Foaming in fermentations. Research under a PL 480 grant at the Superior Institute of Health, Rome, Italy, has shown that a relatively small dilution of a filamentous fermentation caused a large drop in viscosity and correspondingly large increase in aeration efficiency, thus indicating that excessive mycelial growth is perhaps deleterious in mold fermentations. As might be expected, bacterial fermentations were more efficient from the standpoint of oxygen absorbed. The results suggest that fermentation efficiency may well be increased by proper balance of cell growth and product formation with less expenditure for power to achieve necessary aeration.

C. Biological Pesticides

1. Biological insecticides for Japanese beetle. A maximum of 1-2 percent sporulation of a selected strain of Bacillus popilliae has been consistently achieved by 10 day's incubation on a sugar-free solid (agar) medium containing yeast extract and acetate ion. Further work confirmed the absolute repressive effect of glucose and the necessity of acetate for sporulation. Efforts to increase rate and/or extent of spore formation and to obtain sporulation in liquid media have not yet been successful. A small number of these spore structures withstood heat shocking and survivors grew in vitro. They did not infect larvae, perhaps because of the small number of spores in the preparations. Several treatments have been found that increase germination of B. popilliae spores in vitro. The most infective available strains of B. popilliae and B. lenticimorbus have been identified.

Nonvolatile acids found in hemolymph of Japanese beetle larvae include lactic, malic, succinic and glycolic acids. The presence of several others is indicated but not confirmed. Volatile acids found were acetic acid plus small amounts of butyric, propionic and formic acids.

One or more growth factors for Bacillus lenticimorbus and B. popilliae have been found in tap water and agar. When grown in media containing either or both of these materials, B. lenticimorbus withstands endless serial transfer whereas in their absence lyophilized cells survive only two transfers. Growth of B. popilliae is more rapid in media containing these materials. Preliminary experiments indicate that the growth factor or factors may be inorganic in nature. Polarographic measurement of oxygen in growing cultures of B. popilliae showed concentrations during the log phase of growth ranging from 10 to 30 percent of saturation levels.

At Michigan State University evidence has been obtained for metabolism of glucose by B. popilliae via the tricarboxylic acid cycle. Acetate is produced by growing cells and oxidized by resting cells; however, different strains of B. popilliae oxidize acetate at different stages of growth of the cultures. For example, one strain will not oxidize acetate in a culture 24 hours old, whereas another will oxidize acetate at 24 hours and not at 8 hours.

Much information pertinent to the problem of sporulating Japanese beetle pathogens is being accumulated. So far, however, a unifying principle that will explain the significance and relationships of the various observations and lead to solution of the problem has eluded investigators. The limited sporulation on a sugar-free solid medium containing acetate, the discovery of the growth-promoting effects of tap water and agar, and the variable utilization of acetate by different strains suggest that additional emphasis on nutritional requirements may prove fruitful.

2. Insect attractants. Research on screening for insect control agents has been completed. In all, a total of 810 cultures were grown on

grain-based media, and samples were shipped to collaborators in Entomology Research Division for evaluation at five locations. As a result of this decentralized operation and the varying activities of the collaborators, all tests could not be performed as originally planned. The most extensive evaluation of the cultures was as attractants for two species of fruit flies. Review of the tests with all of the collaborators failed to elicit indications that any of the cultures had merit for possible commercial application. Nevertheless, some cultures did perform better than the standards in field tests, and one collaborator said he was considering restudy of two cultures in field tests this summer with Drosophila spp., which are nuisance pests around fruit and berry plantings and processing plants. It is unfortunate that tests on a wider range of insects and for purposes other than attractants were impracticable. However, despite difficulties encountered and the failure to discover products with commercial potential, the results confirm that fermentation cultures do offer possibilities for insect control agents.

3. Plant antibiotics. Of approximately 500 streptomycetes screened to date via shaken-flask fermentations, 196 were found to show only moderate activity against fungi. Ultraviolet absorption studies were conducted with 170 of these and stability studies with 30. Seventy-seven of the 170 were nonpolyenic. Thus far seven strains producing stable and apparently new antifungal antibiotics have been detected.

D. Feed and Food Products

1. Microbial carotenoids. Continuing studies on fermentative production of β -carotene and other carotenoid pigments for use as animal feed supplements have resulted in discovery that dried mycelium of Blakeslea trispora from which carotene and lipid had been extracted is an effective replacement for β -ionone in the fermentation medium. When diphenyl amine is added to a β -carotene fermentation, this metabolic blocking agent causes reduced β -carotene synthesis and accumulation of an intermediate, phytofluene. It may be possible to convert this substance to lutein or zeaxanthin by treatment with other microorganisms. Xanthophyll pigments could be extracted only partially from cell pastes of Sarcina lutea and Micrococcus lysodeikticus. An alga (YB-3399) produced comparatively low concentrations of three xanthophyll compounds but no chlorophyll.

In engineering research, β -carotene has been produced successfully in a 30-gallon pilot-plant fermentation with the organism Blakeslea trispora. A practical recovery procedure for the mycelium, suitable for commercial application, was devised. Demonstration of successful pilot-plant production and recovery of β -carotene should assist in commercialization of the process. Although yields were lower than achieved in the laboratory, the results show that important engineering problems have been solved, and provide a sound basis for further process development.

Results of initial feeding tests at Michigan State University showed that fermentation β -carotene appeared to be as effective as vitamin A in promoting growth but somewhat less effective in replenishing the vitamin A stores of poultry and swine. Analyses performed at the Northern Division showed that there was some loss of β -carotene in the feed mixtures during the tests. Considerable difficulty was experienced in analyzing the comparison feeds, which contained low levels of vitamin A. Results of this first series of feeding tests should not be regarded as conclusive, since the influence of instability of β -carotene and vitamin A in the rations has not been clearly elucidated, in part because of these analytical difficulties. Also, the total data show a need for more rigorous control of preconditioning of test animals and of dosage with materials under test.

2. Vitamin B₁₃. In research under a PL 480 grant at the "Giuliana Ronzoni" Scientific Institute of Chemistry and Biochemistry, Milan, Italy, a method has been developed for extracting and purifying a potent growth factor for mice from distillers' solubles. Mevalonic acid is one of the components of the fraction and appears to be responsible for part of the activity of the factor. However, it is probably not the only active component. Evidence was obtained that orotic acid, thought by some workers to be part of the B₁₃ complex, was not a component of the factor. The present results may constitute the first demonstration that mevalonic acid promotes growth of the animal body.

PUBLICATIONS AND PATENTS REPORTING RESULTS
OF USDA AND COOPERATIVE RESEARCH

Basic Research on Fermentation Processes

Benjamin, C. R., Hendershot, W. F., and Hesseltine, C. W. Nov. 13, 1962.
Fermentative process of producing lactone of 2-oxo-6(2-hydroxypropyl)-cyclohexane carboxylic acid. U. S. Patent 3,063,909.

Benjamin, C. R., Hendershot, W. F., and Hesseltine, C. W. Feb. 26, 1963.
The lactone of 2-oxo-6-(2-hydroxypropyl)-cyclohexanecarboxylic acid. U. S. Patent 3,079,401.

Ellis, J. J. 1963. A study of Rhopalomyces elegans in pure culture. Mycologia 55(2), pp. 183-198.

Galmarini, O. L., Stodola, F. H., Raper, K. B., and Fennell, D. I. 1962. Fonsecin, a naphthopyrone pigment from a mutant of Aspergillus fonseciae. Nature 195(4840), pp. 502-503.

Haynes, W. C. 1963. Discussion II. On the Organization of a Type Culture Collection, by J. M. Shewan, Torry Research Station, Aberdeen, Scotland. In "Culture Collections: Perspectives and Problems," ed. S. M. Martin, University of Toronto Press. Proc. Specialists' Conf. on Culture Collections, Aug. 1962, Ottawa, pp. 36-39.

Haynes, W. C., and Rhodes, L. J. 1962. Comparative taxonomy of crystallo-genic strains of Pseudomonas aeruginosa and Pseudomonas chlororaphis. J. Bact. 84(5), pp. 1080-1084.

Hesseltine, C. W., Benjamin, C. R., Bradle, B., and Hendershot, W. F. 1963. Ramulosin fermentation. Am. J. Botany 50(3), pp. 209-213.

Lyons, A. J., Jr., and Pridham, T. G. 1962. A taxonomic study of some strains of Streptomyces griseus (Krainsky) Waksman et Henrici. Bacteriol. Proc. 1962, p. 41.

Maister, H. G., Rogovin, S. P., Stodola, F. H., and Wickerham, L. J. 1962. Formation of extracellular sphingolipids by microorganisms. IV. Pilot-plant production of tetraacetylphytosphingosine by Hansenula ciferrii. Appl. Microbiol. 10(5), pp. 401-406.

Newton, J. W. 1962. Evidence for a functional disulphide in photophosphorylation. Nature 195(4839), pp. 349-350.

Newton, J. W. 1962. A disulfide photoreduction system in chromatophores of Rhodospirillum rubrum. J. Biol. Chem. 237(10), pp. 3282-3286.

Newton, J. W. 1963. Ionic effects on the photochemical activity of bacterial chromatophores. Proc. Nat. Acad. Sci. 49(6), pp. 866-870.

Pridham, T. G. 1962. Characterization of streptomyces and streptoverticillia and a species-subspecies concept. Bacteriol. Proc. 1962, p. 41.

Pridham, T. G., and Lyons, A. J., Jr. 1962. Proposal to designate strain ATCC 3004 (IMRU 3004) as the neotype strain of Streptomyces albus (Rossi Doria) Waksman and Henrici. Intern. Bull. Bacteriol. Nomenclature and Taxonomy 12(3), pp. 123-126.

Stodola, F. H., Wickerham, L. J., Scholfield, C. R., and Dutton, H. J. 1962. Formation of extracellular sphingolipides by microorganisms. III. Triacetyl dihydrosphingosine, a metabolic product of the yeast Hansenula ciferrii. Arch. Biochem. Biophys. 98(1), p. 176.

Wallen, L. L., Benedict, R. G., and Jackson, R. W. 1962. The microbiological production of 10-hydroxystearic acid from oleic acid. Arch. Biochem. Biophys. 99(2), pp. 249-253.

Weimberg, R. 1963. Studies with a constitutive aldose dehydrogenase in Pseudomonas fragi. Biochim. Biophys. Acta 67(3), pp. 349-358.

Weimberg, R. 1963. Oxidation of glucose to γ - and δ -gluconolactone by microbial enzymes. Biochim. Biophys. Acta 67(3), pp. 359-365.

Weimberg, R. (with the technical assistance of W. L. Orton). 1962. Mode of formation of D-arabitol by Saccharomyces mellis. Biochim. Biophys. Res. Commun. 8(6), pp. 442-445.

Wickerham, L. J., and Burton, K. A. 1962. Phylogeny and biochemistry of the genus Hansenula. Bacteriol. Rev. 26(4), pp. 382-397.

Industrial Chemicals

Gilbert, M. M., Roth, W. B., and Wiebe, R. 1961. High-compression tractor engines with alcohol-gasoline blends. Philippine J. Sci. 90(2), pp. 239-248.

Jeanes, A., Pittsley, J. E., Watson, P. R., and Sloneker, J. H. 1962. Hydrolysis products of periodate-oxidized phosphomannan Y-2448. Can. J. Chem. 40(12), pp. 2256-2259.

Jeanes, A. R., and Sloneker, J. H. Sept. 18, 1962. Atypically salt-responsive alkali-deacetylated polysaccharide produced by Xanthomonas campestris. U. S. Patent 3,054,689.

Jeanes, A., and Watson, P. R. 1962. Periodate-oxidized phosphomannan Y-2448: Structural significance of its reaction with alkali. *Can. J. Chem.* 40(7), pp. 1318-1325.

Orentas, D. G., Sloneker, J. H., and Jeanes, A. 1963. Pyruvic acid content and constituent sugars of exocellular polysaccharides from different species of the genus Xanthomonas. *Can. J. Microbiol.* 9(3), pp. 427-430.

Shono, T., and Marvel, C. S. (University of Arizona, Tucson). 1963. Polymers derived from unsaturated esters of hydronopoxyalkanols. *J. Polymer Sci. (Part A)*, 1, pp. 1543-1552.

Shono, T., and Marvel, C. S. (University of Arizona, Tucson). 1963. Preparation and polymerization of vinyl 12-hydroxystearate. *J. Polymer Sci. (Part A)*, 1, pp. 2067-2074.

Slodki, M. E. 1963. Structure of Hansenula capsulata NRRL Y-1842 phosphomannan. *Biochim. Biophys. Acta* 69(1), pp. 96-102.

Slodki, M. E. Apr. 2, 1963. Dispersants comprising phosphoric acid monesters of mannose polymers. U. S. Patent 3,084,105.

Sloneker, J. H., and Jeanes, A. 1962. Exocellular bacterial polysaccharide from Xanthomonas campestris NRRL B-1459. Part I. Constitution. *Can. J. Chem.* 40(11), pp. 2066-2071.

Sloneker, J. H., and Orentas, D. G. 1962. Exocellular bacterial polysaccharide from Xanthomonas campestris NRRL B-1459. Part II. Linkage of the pyruvic acid. *Can. J. Chem.* 40(11), pp. 2188-2189.

Sohns, V. E. 1962. Chemical engineering in fermentative processing. U. S. Dept. Agr. ARS-71-21, October, 20 pp.

Watson, P. R., Pittsley, J. E., and Jeanes, A. 1962. Problems involved in dialyzing low concentrations of inorganic pyrophosphate. *Anal. Biochem.* 4(6), pp. 505-508.

Wiebe, R., Kretschmer, C. B., and McCloud, A. P. 1961. Volatility of alcohol motor fuels. *Philippine J. Sci.* 90(2), pp. 223-237.

Biological Pesticides

Shotwell, O. L., Bennett, G. A., Hall, H. H., VanEtten, C. H., and Jackson, R. W. 1963. Amino acids in the haemolymph of Popillia japonica (Newman) larvae. *J. Insect Physiol.* 9(1), pp. 35-42.

Feed and Food Products

Ciegler, A., Lagoda, A. A., Sohns, V., Hall, H. H., and Jackson, R. W. 1963. Beta-carotene production in 20-liter fermentors. *Bacteriol. Proc.* 1963, p. 22.

Ciegler, A., Nelson, G. E. N., and Hall, H. H. 1963. Enhancement of beta-carotene synthesis by citrus products. *Appl. Microbiol.* 11(2), pp. 128-131.

Ciegler, A., Nelson, G. E. N., and Hall, H. H. Feb. 26, 1963. Extraction of a substance from dry citrus meal which increases β -carotene production by Blakeslea trispora. U. S. Patent 3,079,380.

Ciegler, A., Nelson, G. E. N., and Hall, H. H. 1963. Stimulation of carotenogenesis in Blakeslea trispora by essential citrus oils. Nature 198(4887), pp. 1305-1306.

Ciegler, A., Pazola, Z., and Hall, H. H. 1963. Stimulation of carotenogenesis by microbial cells. Bacteriol. Proc. 1963, p. 22.

Dansi, A., Dal Pozzo, A., Rotta, L., and Zanini, C. (Scientific Institute of Chemistry and Biochemistry, Milan, Italy). 1962. Sui fattori sconosciuti di crescita dei Distiller's dried solubles. I. Accertamento dell'assenza dell'acido orotico nella "Vitamina B₁₃". Boll. Chim. Farm. 101(5), pp. 380-384.*

Dansi, A., Dal Pozzo, A., Zanini, C., and Rotta, L. (Scientific Institute of Chemistry and Biochemistry, Milan, Italy). 1962. Studio preliminare sulle proprieta del fattore di crescita denominato "Vitamina B₁₃". Chim. Ind. (Milan) 44(8), pp. 839-844.*

Hall, H. H. 1962. Applied microbiology in animal nutrition. Advan. Appl. Microbiol. 4, pp. 77-99.

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AREA NO. 6: FLAXSEED
INDUSTRIAL UTILIZATION OF LINSEED OIL

Problem. Traditional markets for linseed oil, the major drying oil produced and used in the United States, are threatened by widespread use of synthetic products derived from nonagricultural sources. Thus, over the years 1950-1960, use of linseed oil in drying oil products decreased from 590 to 351 million pounds because of displacement by synthetic materials capable of better performance. During the same period, consumption of synthetic products in protective coatings increased by 50 percent.

To restore the competitive position of linseed oil, new or expanded markets are urgently needed. Such markets can be achieved by an adequate program of basic and applied research. Recent studies by Department scientists have resulted in commercial manufacture and sale of linseed emulsion paints for exterior use that are competitive with synthetic resin emulsion paints. Use of these new linseed oil paints is expected to expand and assist in maintaining linseed oil in the market for exterior paints, which amounted to 70-75 million gallons in 1962. Another new product from linseed oil to which Department research is contributing is a protective coating for concrete that prevents deterioration from deicers and freezing and thawing in winter. Indications are that use of these two new products may halt the decline in consumption of linseed oil. However, additional research is needed to insure maximum acceptance and consumption of these new coatings and to provide still other new or improved products from linseed oil that can maintain and increase its use in all types of protective coatings, a market exceeding 640 million gallons in 1962.

Other new outlets can be realized by chemical modification of linseed oil to obtain materials that will find applications in the multibillion-pound annual market for products of the organic chemical industry. To furnish a sound basis for chemical modification, a broad program of basic research on linseed oil is required to furnish new leads and new concepts that will point the way to those products having the best chance for acceptance.

USDA PROGRAM

The Department conducts a continuing long-range program involving analytical, organic and physical chemists and chemical engineers engaged in basic research and on the chemical reactions of linseed oil and its component fatty acids and in the application of the knowledge gained to the development of new or improved products for the chemical and protective coating industries.

The Federal scientific effort concerned with research on industrial uses for linseed oil totals 21.8 professional man-years. Of this number 6.0 is devoted to industrial chemical products and 15.8 to protective coating products.

The current program at Peoria, Illinois, does not include research specifically devoted to chemical composition and physical properties.

Research at Peoria, Illinois, on industrial chemical products (6.0 professional man-years) involves exploratory studies to find new reactions and chemical derivatives and basic and applied research on cyclic fatty acids. During the reporting period contract research to identify promising applications for aldehyde products from linseed oil was completed.

Studies on protective coating products in progress at Peoria, Illinois, (14.4 professional man-years) include investigations on new polymers from linseed oil for use as water-soluble vehicles for coatings and basic and applied research on problems related to development of linseed emulsion paints. During the reporting period research was discontinued on linseed vinyl ethers and their use as protective coatings, except for provision of samples to interested industrial companies. Research contracts on protective coating products (1.4 professional man-years) are in effect with the University of Southern California, Los Angeles, California, for basic physical chemical studies on linseed oil emulsions and pigment suspensions (.7 professional man-year) and with Kansas State University, Manhattan, Kansas, for research on the use of linseed oil to protect concrete (.7 professional man-year).

The Department also sponsors research conducted by foreign institutions under grants of PL 480 funds. Research on chemical composition and physical properties involves a grant to the Experiment Station for Fats and Oils, Milan, Italy, for studies on minor constituents of linseed oil (5 years, 1960-1965). Research on industrial chemical products is conducted by this institution also under a grant for the investigation of products obtained by thermal polymerization of linseed and other polyunsaturated vegetable oils (4 years, 1960-1964) and by the Regional Research Laboratory, Hyderabad, India, under a grant for exploratory studies on hydroxylation of linseed and safflower oils (5 years, 1963-1968). Research on protective coating products involves a grant to the Paint Research Station, Teddington, England, for fundamental research on organometallic compounds as components of protective coatings (5 years, 1960-1965).

REPORT OF PROGRESS FOR USDA AND COOPERATIVE RESEARCH

A. Chemical Composition and Physical Properties

1. Minor constituents of linseed oil. Progress continues to be made on the isolation and identification of the components of the unsaponifiables of linseed oil. Although the substance responsible for the positive Fitelson reaction in tea seed oil has been tentatively identified as butyrospermol and several substances of similar structure have been shown to give a positive test, the identity of the Fitelson positive component or components in linseed unsaponifiables is still uncertain. Linseed sterols were found to comprise β -sitosterol, stigmasterol and an unidentified

sterol. Chromatography of unsaponifiables from six different samples of U. S. linseed oil revealed qualitative differences in composition. The work thus far has yielded information on the nature of the linseed unsaponifiables that should be useful in increasing the utilization of linseed oil in industrial products. This research is being performed by the Experiment Station for the Fats and Oils Industry, Milan, Italy, under a PL 480 grant.

B. Industrial Chemical Products

1. Cyclic acids. It has been discovered that when C_{18} cyclized monomer is improperly hydrogenated over a palladium catalyst, both saturated (cyclohexane type) and aromatic cyclic acids are produced in varying proportions depending on conditions. Detailed studies revealed reaction conditions that permit high conversions of cyclic acids either to pure aromatized acids or to hydrogenated cyclic acids free of aromatic product. Addition of a substituted ethylene to a conjugated dienoic acid was found to be much more difficult than that of ethylene itself. Thus with 9,11-t, t-octadecadienoic acid, ethylene gave a 92-percent yield of product at 260° , whereas isobutylene gave no adduct at 260° but did give a 35-percent yield at 295° . (C_{18} materials result from alkaline cyclization; C_{20} materials from ethylene addition.)

Liquid-liquid extraction tests on monomeric fatty acids from the alkaline cyclization of linseed oil showed that cyclic acid content could be increased from 45 percent in the feed to 80 percent in the extract. However, to displace the more costly low-temperature crystallization method, purity approaching 100 percent must be achieved. Other studies showed that 99-percent recovery of ethylene glycol, the solvent for cyclization, could be attained. The "cost-to-make" for hydrogenated cyclic acid has been reestimated on the basis of present knowledge to be 40 cents per pound for a plant producing 4 million pounds per year. The "cost-to-make" for crude cyclic acid mixture (contains cyclic acid, unreacted fatty acids and some polymer) is estimated at 20 cents and for polymer-free mixture, 22.5 cents.

Fatty alcohols have been prepared from several types of cyclic acid products. Evaluations by an industrial company indicate promise for certain of these alcohols as components of cosmetics. Vinyl esters of hydrogenated C_{18} cyclic acids were obtained by direct vinylation in yields of over 90 percent of once-distilled product.

2. Glyceride polymers. In research under a PL 480 grant at the Experiment Station for the Fats and Oils Industry, Milan, Italy, valuable information has been obtained on polymerized linseed oil by studying the structure of the glycerides and the acids obtained therefrom. Evaluation of the data collected thus far has enabled the investigators to deduce some of the reactions involved in heat polymerization of linseed oil. For example, the initial step appears to be dimerization of the glyceride. Two dimers then

react to form tetramers which undergo further polymerization reactions. This information may be useful in preparing better commercial products of this type, thus increasing the utilization of linseed oil.

C. Protective Coating Products

1. Emulsion paints. Studies have shown that a change of 0.1 HLB unit (HLB is a numerical measure of the tendency of surfactants to produce oil-in-water emulsions as compared to water-in-oil emulsions) may be sufficient to decrease the stability of ZnO-containing emulsion paints. Reactivity of ZnO was decreased by treating it with ethylene glycol before adding other ingredients of the pigment dispersion. Extensive studies of adsorption of phosphates on zinc and titanium oxides have led to a correlation between monolayer adsorption and minimum requirement for viscosity stability. A new technique which uses inversion with slow-speed stirring gave dispersions of small particle size (ca. 1 micron) from high-viscosity, bodied linseed oils. Based on these studies, a series of emulsion paints designed to compare linseed oils having different degrees of polymerization and to investigate the effect of inorganic phosphate dispersant on mildewcidal properties of zinc oxide has been prepared. Three member companies of the NFPA will conduct and evaluate outdoor exposure tests and tests of application properties of these paints.

At the University of Southern California experimental phases of contract research on emulsion paints have been completed. In the concluding work a method was developed for determining the total interfacial area of oil-in-water emulsions. Stability was shown to be greater for emulsions with larger interfacial areas and for those having the larger fraction of the interfacial area covered with stabilizer (sodium dodecyl sulfate). Further application of the ultracentrifugal method for quantitative measurement of emulsion stability involved study of several variables important to practical paint systems. For example, it was shown that increasing the volume fraction of oil in an emulsion increased stability. Studies on drop-size distribution of M-37 linseed oil emulsions showed that smaller drops were formed in the presence of small percentages of ZnO and stability of the emulsions decreased.

Research on linseed emulsion paints continues to provide information important to the continued development of these paints on a commercial basis. Three companies now make linseed emulsions available to paint manufacturers. A fourth company is marketing a 100-percent solids product for dispersal in water by the user, and a fifth is marketing a water-soluble vehicle based on linseed oil. A recent report indicates that consumption of water-based exterior paints shows a growth rate approaching three times that of the paint industry as a whole. The increasing number of companies marketing linseed emulsion paints indicates growing consumer acceptance and suggests that a large share of the expanding market for water-based exterior coatings will be captured by linseed emulsion paints. The basic aspects of our research on linseed emulsion paints are especially important because

they will provide the key to solution of problems that will inevitably arise. For example, one manufacturer of linseed emulsion vehicles has credited the Northern Division research on pigment interaction with solution of a problem encountered in pigmenting the emulsion. Furthermore, the basic research on emulsion paints as well as that on water-soluble vehicles and on new polymers for coatings will provide the foundation for improved products to meet future competition.

2. Linseed coatings for concrete. At Kansas State University preparation of suitable concrete slabs, as specified in the contract, has been completed and the freeze-thaw phase of work has begun. A stable 50-percent emulsion of boiled linseed oil was developed at the Northern Division for use in this research in addition to solutions of linseed oil in mineral spirits. The objective of this work is to obtain precise information on the value of linseed oil in protecting air-entrained concrete from deterioration caused by freezing and thawing with application of deicers in winter. A growing number of states are using linseed oil in quantity for coating highways or are experimenting with it. Preliminary laboratory and field results indicate that emulsified boiled linseed oil can be used as a curing compound for concrete. On the basis of 1962 highway construction, the potential market for linseed oil on highways is 50 million pounds per year. Use on other concrete structures such as dams, buildings, etc., could increase this potential market considerably.

3. Water-soluble and other new vehicles based on linseed oil. Good progress is being made in the initial phases of recently undertaken research designed to develop new water-soluble and other improved vehicles and coatings resins from linseed oil. A vehicle that is soluble in aqueous isopropanol, sets to touch in 4 hours and dries hard and tack-free in 16 hours was prepared from tris(hydroxymethyl)aminomethane, itaconic acid, linseed fatty acids and dimethylaminoethanol. Attempts to prepare dilinseed esters of α -methyl or α -allyl glucoside by direct esterification or by alcoholysis of linseed methyl esters gave a mixture of products. These have been tentatively identified as the tetramethyl ester, several triesters, including the 2,4,6-triester, the 2,3-diesters and the 3- and 6-monoesters.

Procedures have been developed for preparation in high yields and purity of the monolinseed ester of ethylene glycol and the hydroxyethyl amide of linseed fatty acids. These products are intermediates for study in preparation of new types of vinyl monomers.

4. Organometallic compounds in paints. A series of novel organometallic derivatives has been prepared by reaction of long-chain unsaturated acetoacetates with aluminum or titanium alkoxides. Several of the products showed promising properties as coatings. Compatible mixtures with alkyd resins were prepared that behaved well with respect to accelerated weathering and water-resistance and that showed improved adhesion to mild steel in comparison to the original alkyd. Derivatives of gallates formed from

castor oil and a castor oil modified alkyd showed promise as primers for mild steel. Studies on interactions of pigments and coating media indicated that titanium dioxide adsorbs a fatty acid in part, and perhaps wholly, by an ionic mechanism. This work is being performed by the Paint Research Station, Teddington, England, under a PL 480 grant.

PUBLICATIONS AND PATENTS REPORTING RESULTS
OF USDA AND COOPERATIVE RESEARCH

Industrial Chemical Products

Beal, R. E. Sept. 4, 1962. Preparation of polyunsaturated fatty acids. U. S. Patent 3,052,699.

Cowan, J. C. 1962. Dimer acids. J. Am. Oil Chemists' Soc. 39(12), pp. 534-545.

DeJarlais, W. J., and Teeter, H. M. 1962. Nitrogenous derivatives of cyclic fatty acids. J. Am. Oil Chemists' Soc. 39(10), pp. 421-424.

Eisenhauer, R. A., Beal, R. E., and Griffin, E. L., Jr. 1963. Cyclic fatty acid yields from linseed oil. J. Am. Oil Chemists' Soc. 40(4), pp. 129-131.

Fedeli, E., Arpino, A., and Capella, P. (Experiment Station for the Fats and Oils Industry, Milan, Italy). 1963. Determinazione mediante il metodo isopiesticco del peso molecolare di oli termopolimerizzati e di loro frazioni. Chim. Ind. (Milan) 45(2), pp. 208-211.*

Friedrich, J. P., and Beal, R. E. 1962. Liquid C-18 saturated monocarboxylic acids--their preparation, characteristics, and potential uses. J. Am. Oil Chemists' Soc. 39(12), pp. 528-533.

Friedrich, J. P., Bell, E. W., and Beal, R. E. 1962. Reaction of ethylene with C₁₈ monocarboxylic dienoic acids. J. Am. Oil Chemists' Soc. 39(9), p. 420.

Marvel, C. S.,¹ Dykstra, T. K.,¹ and Magne, F. C.² (University of Illinois, Urbana; ²South. Util. Res. Devlpmt. Div., Agr. Res. Serv., U.S. Dept. Agr., New Orleans, La.). 1962. Some polymers and copolymers of vinyl keto-stearates. J. Polymer Sci. 62(174), pp. 369-377.

Marvel, C. S., and Kiener, P. E. (University of Illinois, Urbana). 1962. Polyallyoocimene. II. J. Polymer Sci. 61(172), pp. 311-331.

Sohns, V. E., Beal, R. E., and Griffin, E. L., Jr. 1963. Cost of producing linoleic acid from safflower oil. J. Am. Oil Chemists' Soc. 40(5), pp. 169-172.

Protective Coating Products

Cowan, J. C. 1962. Research on linseed oil at Northern Regional Research Laboratory. Proc. 32nd Annual Flax Inst. U.S., Minneapolis, Minn., pp. 26-28.

Cowan, J. C., and Gast, L. E. 1963. Research on emulsion paints from vegetable oils. Am. Paint J. 47(33), pp. 85,88,89,92,94,96,98,100, 102,104.

*Research supported by PL 480 funds.

Gast, L. E., Schneider, W. J., Teeter, H. M., McManis, G. E., and Cowan, J. C. 1963. Reactions of unsaturated fatty alcohols. XV. Styrenation of fatty vinyl ether polymers in terpene solvents. J. Am. Oil Chemists' Soc. 40(3), pp. 88-91.

Kubie, W. L., O'Donnell, J. L., Teeter, H. M., and Cowan, J. C. 1963. Emulsifiers derived from linseed oil and their potential use in coatings. J. Am. Oil Chemists' Soc. 40(3), pp. 105-107.

Kubie, W. L., Stolp, J. A., Schwab, A. W., Teeter, H. M., and Cowan, J. C. 1962. Linseed oil emulsion paints having increased stability to driers and pigments. Offic. Dig. Federation Soc. Paint Technol. 34(450), pp. 693-703.

Kubie, W. L., Stolp, J. A., Schwab, A. W., Teeter, H. M., and Cowan, J. C. 1963. Linseed oil emulsion paints having increased stability to driers and pigments. J. Appl. Chem. 13(5), p. 611.

Mayo, F. R., and Huff, H. (Stanford Research Institute, Menlo Park, Calif.). 1963. Polymerization and copolymerization of unsaturated fatty acids and their esters--a survey of the literature. North. Util. Res. Devlpmt. Div., Agr. Res. Serv., U.S. Dept. Agr. ARS-71-30, 48 pp.

Princen, H. M. (University of Southern California, Los Angeles). 1963. Shape of a fluid drop at a liquid-liquid interface. J. Colloid Sci. 18(2), pp. 178-195.

Princen, L. H., and DeVena, M. J. 1963. Reactivity of zinc oxide and titanium dioxide in the presence of dispersing agents. J. Am. Oil Chemists' Soc. 40(4), pp. 131-135.

Schwab, A. W., and Teeter, H. M. July 31, 1962. Fast surface-dry oil emulsion paint. U. S. Patent 3,047,413.

Teeter, H. M. 1963. Vinyl monomers derived from fats and oils. J. Am. Oil Chemists' Soc. 40(4), pp. 143-156.

Vold, R. D., and Groot, R. C. (University of Southern California, Los Angeles). 1962. An ultracentrifugal method for the quantitative determination of emulsion stability. J. Phys. Chem. 66(10), pp. 1969-1975.

Vold, R. D., and Groot, R. C. (University of Southern California, Los Angeles). 1963. Parameters of emulsion stability. J. Soc. Cosmetic Chemists 14(5), pp. 233-244.

Vold, R. D., and Singh, H. (University of Southern California, Los Angeles). 1962. Reactions between zinc chloride and surfactant solutions. J. Am. Oil Chemists' Soc. 39(10), pp. 424-430.

General

1962. Review articles on oilseed crops research. A list of publications, 1936-1961. North. Util. Res. Devlpmt. Div., Agr. Res. Serv., U.S. Dept. Agr. ARS-71-29, 9 pp.

1963. Review articles on oilseed crops research. A list of publications for 1962. North. Util. Res. Devlpmt. Div., Agr. Res. Serv., U.S. Dept. Agr. ARS-71-29-1, 1 p.

AREA NO. 7: SOYBEANS
FOOD AND INDUSTRIAL USES FOR SOYBEAN OIL

Problem. Soybean oil is now the major edible oil of the United States and the most important source of nutritionally important linoleic acid. However, this oil contains an unstable component (linolenic acid) that limits its use as a liquid oil both domestically and in foreign markets. It is estimated that in 1962 at least 3.4 billion pounds of soybean oil (about 90 percent of total domestic use) was consumed in edible products, of which somewhat more than two-thirds was consumed in hydrogenated form as margarine and shortening. However, production of soybeans continues to increase rapidly and exceeded 670 million bushels in 1962.

The most promising outlets for oil from this ever-growing production of soybeans appear to be in foreign markets as edible oils and fats and in domestic industrial uses. The potential market for vegetable oils imported by Europe is estimated at 7.5 billion pounds by 1975. For soybean oil to capture a growing share of this market, more information is needed to show how to eliminate unstable linolenic acid without loss of nutritive value, to determine the extent to which minor constituents influence flavor and other properties of the oil, and to discover methods for modifying hydrogenated soybean oil to achieve desired functional properties such as melting point and texture. This information would also serve as the basis for improving soybean oil for domestic use both as a liquid oil and in its hydrogenated forms. Some additional consumption in the United States might be anticipated because of extended utility resulting from these improvements, even though consumption of edible fats and oils mainly increases with population growth. To achieve the objective, a broad program of basic and applied research is required to provide more knowledge of the properties of linolenic acid and of minor constituents of soybean oil; of the changes that take place in these and other components during oxidation, hydrogenation, and heating; of the effects of these changes on flavor, nutritive value, stability, and other qualities of the oil; and of the effects of modification of glyceride structure on functional properties of hydrogenated forms of soybean oil.

As an industrial oil, soybean, like linseed oil, is faced with growing competition from synthetic products derived from nonagricultural sources. As an industrial source of linoleic acid, soybean fatty acids must also compete with tall oil fatty acids, a byproduct of paper manufacture. The best opportunity for increasing industrial applications of soybean oil appears, therefore, to be development of products that retain the glyceride structure of the oil. Thus, aldehyde oils, a recent discovery of Department scientists, appear to have a promising future, if current research and development is successful, in the 3-billion-pound market for resins, fibers, coatings, plastics, plasticizers, pesticides, and paper and textile chemicals. To achieve the potential industrial value of aldehyde oils and other

soybean glyceride products, more fundamental information is needed on reactions of soybean oil that will preserve the glyceride structure and on the physical and chemical properties of the products. Upon this basis, development of a wide variety of new, industrially useful products should be possible.

USDA PROGRAM

The Department has a continuing long-range program involving analytical, organic and physical chemists and chemical engineers engaged in basic and applied research on edible and industrial uses of soybean oil. A food technologist is also required by the program in connection with organoleptic evaluation of edible oils. Objectives of research on edible soybean oil are to identify undesirable flavor components of the oil, to develop basic information on the chemical changes and mechanisms involved in formation or suppression of these components and to apply the knowledge gained to the development of edible soybean oil having improved oxidative, thermal and organoleptic stability. Objectives of research on industrial utilization are to obtain new information on reactions of soybean oil and its components and to use this information to develop new or improved products for use by the chemical and other industries.

The Federal scientific effort for research on soybean oil totals 31.4 professional man-years. Of this number 9.0 are devoted to chemical composition and physical properties, 11.4 to edible utilization, and 11.0 to industrial utilization.

Research at Peoria, Illinois, on chemical composition and physical properties (9.0 professional man-years) is concerned with isolation and identification of components affecting flavor stability of soybean oil. During the reporting period basic studies on autoxidation of soybean oil were completed.

Research at Peoria, Illinois, on edible utilization of soybean oil (8.5 professional man-years) involves basic and applied studies on selective hydrogenation and on interesterification followed by selective extraction as means of stabilizing soybean oil by removal of linolenate. Studies are also in progress on the stability of mixtures of soybean oil with other edible oils. Research contracts (2.9 professional man-years) are in effect at Armour Research Foundation, Chicago, Illinois, for development of heterogeneous selective hydrogenation catalysts (1.4 professional man-years); at Rutgers, The State University, New Brunswick, New Jersey, for basic studies on heterogeneous catalysts (1.0 professional man-year); and at the University of Illinois, Urbana, Illinois, for basic research on homogeneous catalysts (.5 professional man-year). During the reporting period a contract project was completed that covered preparation and evaluation of several types of heterogeneous catalysts.

Research at Peoria, Illinois, on industrial utilization of soybean oil (10.6 professional man-years) involves exploratory studies to find new reactions and products and basic and applied investigations of aldehyde oils and other aldehydic products. A research contract (.4 professional man-year) is in effect with North Dakota State University of Agriculture and Applied Science for investigations of aldehyde oils as components of protective coatings. During the reporting period contract research to identify promising applications for aldehyde products from soybean oil was completed.

The Department also sponsors research on soybean oil conducted by foreign institutions under grants of PL 480 funds. Research on chemical composition and physical properties involves grants to the Institute for Fats and Their Derivatives, Seville, Spain, for research on removal of trace metals from soybean oil with ion-exchange resins (5 years, 1960-1965) and to Gdansk Polytechnic, Gdansk, Poland, for studies on soybean sterols and their effect on stability of the oil (4 years, 1961-1965). Research on edible utilization is conducted under grants to the University of Granada, Spain, for studies on the effect of processing on frying quality of soybean oil (5 years, 1962-1967) and to Tokyo University, Japan, for research on hydrogenation of soybean oil (3 years, 1962-1965). Research on industrial utilization involves grants to the University of Helsinki, Finland, for studies on separation of pure fatty acids from mixtures such as soybean fatty acids (5 years, 1960-1965); Queen Mary College, University of London, England, for basic studies on alkaline cleavage of polyunsaturated fatty acids (4 years, 1960-1964); and the Experiment Station for the Fats and Oils Industry, Milan, Italy, for research on oxidation with atmospheric oxygen to obtain new soybean oil derivatives (4 years, 1960-1964).

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition and Physical Properties

1. Flavor components. The hydrocarbon portion, which comprises 10 to 15 percent of the unsaponifiables of soybean oil, was shown to be primarily responsible for flavor deterioration caused by unsaponifiables. Squalene constituted 50 percent of the mixture and contained most of the unsaturation. Some unsaturation was observed in two compounds of C_{23} and C_{26} carbon chain length. A crystalline portion, constituting 4.2 percent of the hydrocarbons, was composed primarily of C_{29} and C_{31} saturated hydrocarbons, with some C_{27} and C_{30} being present. The remainder was a very complex mixture of C_{15} to C_{35} hydrocarbons. About 1 percent of unsaponifiables is present in soybean oil.

New analytical techniques, which involve a combination of hydrolysis with lipase and chromatographic analysis of the monoglycerides so produced, were developed and used to demonstrate and confirm the postulated intramolecular random arrangement of unsaturated fatty acid groups in soybean and other oils.

2. Removal of prooxidant metals. In studies under a PL 480 grant at the Institute for Fats and Their Derivatives, Seville, Spain, efficient removal of prooxidant metals from soybean oil has been achieved by use of ion-exchange resins. Organoleptic tests showed that this removal resulted in improvement in flavor stability statistically significant at the 5- and 1-percent levels.

3. Effects of sterols on flavor stability. Experiments at Gdansk Polytechnic, Gdansk, Poland, showed that chemical changes in soybean sterols result from the action of bleaching earths. Soybean sterols and their alteration products are being characterized by chromatographic and spectrophotometric techniques. Results suggest that steroid compounds with increased unsaturation caused by dehydration are formed. This research is being conducted under a PL 480 grant.

B. Edible Utilization

1. Selective hydrogenation. In basic studies on kinetics and mechanism of hydrogenation, several important advances were made: (1) anticipated isotopic effects were not observed in catalytic reduction of methyl oleate with hydrogen-deuterium-tritium gas mixtures; (2) extensive exchange of deuterium for carbon-bonded hydrogen was shown to occur during catalytic reduction of methyl oleate with deuterium; (3) in analogous reductions of methyl 9,10-ditritiooctadec-9-enoate, tritium was not released during saturation of the double bond but appears upon completion of the reduction; (4) homogeneous catalytic hydrogenation of sodium sorbate with pentacyanocobaltate II as catalyst gave 80-, 19- and 1-percent yields of 2-, 3- and 4-hexenoic acids; (5) a small analog computer was assembled and applied successfully to kinetic problems of selective hydrogenation.

Iron pentacarbonyl was found to be an effective homogeneous catalyst for hydrogenation of soybean oil and its methyl esters. However, there was no selectivity towards linolenate, and isomerization occurred with accumulation of trans-containing dienes (conjugated) and monoenes. There was little or no increase in saturates. In heterogeneous hydrogenation of methyl linolenate, platinum was less selective than commercial nickel catalyst, but there was less bond migration and trans formation. A rapid quantitative method was developed for use of nuclear magnetic resonance in determining the 15,16 double bond content of lipids.

Studies of the hydrogenation-winterization process showed that changes in fatty acid composition, trans-acid formation, and yield of winterized oil were approximately linear with degree of hydrogenation.

In final phases of contract research at Armour Research Foundation, catalysts consisting of nickel supported on molecular sieves showed selectivities for linolenic over linoleic as high as 2.9, but selectivities at least as high as 8 may be needed for commercial use. Trans isomers were in the range of 4 to 13.8 percent, and better results were obtained at

65-80° C. than at 100-150°, the temperature used with conventional supported nickel catalysts.

2. Improving flavor stability. Flavor scores of soybean, cottonseed and partially hydrogenated soybean oils exposed to fluorescent light for periods of 1 to 4 hours have been found to correlate well with those for oils submitted to oven storage (4 days required) and the A.O.M. test (8 hours required). Safflower oil, even though it is highly unsaturated and does not have high heat stability, showed surprising stability to fluorescent light. Heating of degassed oils under vacuum was found to be very deleterious to flavor. When oils of differing stability were mixed, the stability of the mixture was observed to vary linearly with composition.

The rapidity and convenience of testing by exposure to fluorescent light will expedite studies planned on flavor stability. Furthermore, this type of test, by providing information on factors contributing to light instability, can contribute to solution of problems arising from packaging of oils in clear glass bottles. Observation of the unusual light stability of safflower oil is very significant, since it has commonly been believed that stability of oils became greater as their iodine number decreased. It is now evident that other factors are operative. Elucidation of these factors should make possible further important advances in stabilizing liquid soybean oil.

3. Frying quality of soybean oil. Preliminary taste panel tests have shown that when potatoes are fried in soybean oil, olive oil, or a 50-50 mixture of soybean and olive oil, it is impossible to distinguish the type of oil used. Since these results were based on oil used for one frying operation only, it has been suggested that further studies be made on continued or repeated use of the oil in deep-fat frying. This research is being conducted by the University of Granada, Granada, Spain, under a PL 480 grant.

4. Partial hydrogenation of soybean oil. Initial experiments under a PL 480 grant at Toyo University, Kawagoe, Saitama-ken, Japan, showed that Cu-Cr-catalyst and Cu-Cr-Mn-catalyst were effective for "selective" hydrogenation of soybean oil at comparatively low temperatures and small amounts of catalysts. The meaning of "selective" hydrogenation was not given but it is presumed to mean hydrogenation to monoenes. Cu-Ni-catalysts were ineffective but their preparation will be studied further.

C. Industrial Utilization

1. Oxidative cleavage of soybean oil and its fatty acids. By use of the bisulfite adduct, methyl azelaaldehydate of 99.8-percent purity can be obtained. Experiments on partial ozonization of methyl linoleate and linolenate indicated that attack by ozone was essentially random. The C-12 and C-15 aldehyde esters thus prepared are being characterized.

Procedures have been developed for isolation in 70-percent yields of malonaldehyde (as its tetramethyl acetal) from the ozonization products of polyunsaturated systems. In engineering studies, treatment of aldehyde products with cation exchange resins was found to improve color substantially and to minimize undesired polymerization. The resin, which presumably removes metallic contaminants, can be regenerated and reused.

2. Aldehyde oils and derivatives. Linear elastomeric poly(ester-acetals) have been prepared from methyl azelaaldehydate (MAZ) and glycerol. Another new polymer was obtained by simultaneous hydrolysis and polymerization of isopropylidineglyceryl azelaaldehydate dimethyl acetal under conditions similar to those for interfacial polymerization. Studies point to boron trifluoride as the best catalyst for crosslinking poly(ester-acetal) resins on glass. Coatings required baking at 300° C. to achieve adherent, solvent-resistant films. Five additional ester-acetal derivatives of azelaaldehydic acid were prepared for evaluation at the Eastern Utilization Research and Development Division as plasticizers and stabilizers for vinyl plastics. Vinylation of the methanol and ethylene glycol acetals of azelaaldehydic acid gave the corresponding vinyl esters in about 90-percent yields. These products will be evaluated as internal plasticizers for poly(vinyl chloride).

Contract studies at Battelle have been completed. Acetals of aldehyde oils and of MAZ were shown to react more readily with polyols than did the free aldehyde forms. The reaction product of trialdehyde oil acetal with methyl glucoside was a thermoplastic solid that shows promise as a hot-melt adhesive for nonpolar polymers such as polyethylene. The reaction product from MAZ and dextrose gave a hard film on glass that was insensitive to boiling water. A tough, elastomeric product possibly useful as a plasticizing agent was obtained from poly(vinyl alcohol) and MAZ acetal.

Industrial interest in aldehyde oils and related materials continues to grow. Recently a semicommercial quantity of MAZ was produced by an industrial company at the request of a leading manufacturer of resins and plasticizers. A third company is considering pilot-plant production. Prospects appear good for ultimate commercialization of MAZ. However, progress in finding promising uses for aldehyde oils has been slow. Because of the unusual chemical nature of aldehyde oils, basic studies should be emphasized in order to develop new information on their properties and reactions that can assist in finding industrial outlets for these products.

3. Separation of fatty acids. Research under a PL 480 grant at the University of Helsinki, Finland, has shown that crystallization of soybean oil and linseed oil fatty acid-fatty acid soap mixtures from methanol solution at low temperatures yields fractions containing over 90 percent of polyunsaturated fatty acids. Solvent requirements were not excessive, but recoveries of polyunsaturated acids were low. Separation of linoleic acid from linolenic acid, contained in concentrates, was not feasible at practical temperatures.

4. New derivatives. Alkali fusion of hydroxyketo fatty acids where the hydroxy- and keto-groups are adjacent and removed from the carboxylic acid group gave excellent yields of dibasic acids by cleavage between the keto- and hydroxy-groups. Studies supported the hypothesis that alkali fusion of unsaturated fatty acids proceeds through movement of the double bond to the alpha or two position in the chain. These studies are in progress under a PL 480 grant to Queen Mary College, University of London, England.

Under a PL 480 grant to the Experiment Station for the Fats and Oils Industry, Milan, Italy, metal chelates of Cu^{II}, Co^{II}, Ni^{II}, Fe^{II}, Fe^{III}, and Zn^{II} with Schiff bases derived from salicyl aldehyde and long-chain substituted diamines have been prepared and purified. Methyl oleate and linseed methyl esters have been oxidized with these chelates and with those from the Schiff base of ethylene diamine and salicyl aldehyde ("Calvin chelates"). Initial studies on the separation and identification of methyl oleate oxidation products have been carried out.

PUBLICATIONS AND PATENTS REPORTING RESULTS
OF USDA AND COOPERATIVE RESEARCH

Chemical Composition and Physical Properties

Cowan, J. C., and Evans, C. D. 1962. Flavor reversion and related forms of oxidative deterioration. In "Autoxidation and Antioxidants," ed. W. O. Lundberg, Interscience Publishers, Inc., New York, Vol. II, Chap. 14, pp. 593-628.

Dutton, H. J. 1963. Monitoring gas chromatography for H³- and C¹⁴-labeled compounds by liquid scintillation counting. In "Advances in Tracer Methodology," ed. Seymour Rothchild, Plenum Press, New York, Vol. 1, pp. 147-152.

Dutton, H. J., Jones, E. P., Davison, V. L.,¹ and Nystrom, R. F.¹ (¹University of Illinois, Urbana). 1962. Labeling fatty acids by exposure to tritium gas. III. Methyl stearolate and methyl linolenate. J. Org. Chem. 27(7), pp. 2648-2649.

Dutton, H. J., and Nystrom, R. F.¹ (¹University of Illinois, Urbana). 1963. Reaction of unsaturated organic compounds with tritium gas. In "Advances in Tracer Methodology," ed. Seymour Rothchild, Plenum Press, New York, Vol. 1, pp. 18-27.

Dutton, H. J., and Scholfield, C. R. 1963. Recent developments in the glyceride structure of vegetable oils. In "Progress in the Chemistry of Fats and Other Lipids," eds. R. T. Holman, W. O. Lundberg, and T. Malkin, Chap. 10, Vol. 6, pp. 313-339.

Hoffmann, R. L., Moser, H. A., Evans, C. D., and Cowan, J. C. 1962. Soybean unsaponifiables: Chromatographic separations and characterization. J. Am. Oil Chemists' Soc. 39(7), pp. 323-327.

Edible Utilization

Cowan, J. C. 1962. Polyunsaturates: Domestic supplies and products. *J. Am. Oil Chemists' Soc.* 39(9), pp. 4-5, 14-15.

Dutton, H. J. 1963. Kinetics of linolenate hydrogenation. *J. Am. Oil Chemists' Soc.* 40(1), pp. 35-39.

Dutton, H. J., Scholfield, C. R., Jones, E. P., Pryde, E. H., and Cowan, J. C. 1963. Hydrazine-reduced linolenic acids as a source of C_9 , C_{12} , and C_{15} dibasic acids. *J. Am. Oil Chemists' Soc.* 40(5), pp. 175-179.

Scholfield, C. R., Jones, E. P., Butterfield, R. O., and Dutton, H. J. 1963. Hydrogenation of linolenate. Fractionation of isomeric esters by countercurrent distribution with an argentation system. *Anal. Chem.* 35(3), pp. 386-389.

Sreenivasan, B., Nowakowska, J., Jones, E. P., Selke, E., Scholfield, C. R., and Dutton, H. J. 1963. Hydrogenation of linolenate. VII. Separation and identification of isomeric dienes and monoenes. *J. Am. Oil Chemists' Soc.* 40(2), pp. 45-51.

Industrial Utilization

Miller, W. R., and Cowan, J. C. 1962. Tetracyanoethylene adduct of trans,trans-9,11-octadecadienoic acid. *J. Am. Oil Chemists' Soc.* 39(8), p. 380.

Mustakas, G. C. Apr. 9, 1963. Method of preparing an air-drying conjugated soybean vinyl ether and isobutyl vinyl ether copolymer. U. S. Patent 3,085,079.

Pryde, E. H., Anders, D. E., Teeter, H. M., and Cowan, J. C. 1962. Selective hydrogenation of methyl oleate ozonolysis products by palladium in pyridine-methanol solvent. *J. Org. Chem.* 27(9), pp. 3055-3059.

Pryde, E. H., and Cowan, J. C. 1962. Aldehydic materials by the ozonization of vegetable oils. *J. Am. Oil Chemists' Soc.* 39(11), pp. 496-500.

Uksila, E. (University of Helsinki, Finland). 1963. Fractionation of soybean and linseed oil fatty acids by crystallization. *Ann. Acad. Sci. Fennicae, Ser. A II*(119), 74 pp.*

General

McKinney, L. L. 1962. Oil uses and their potential. *Proc. Pacific Northwest Oilseed Crop Conf.*, Oregon State University, Corvallis, May 15-16, pp. 73-100.

1962. Chemically modified oil products and industrial uses. A list of publications and patents, 1936-1961, Northern Regional Research Laboratory. *North. Util. Res. Devlpmt. Div., Agr. Res. Serv., U.S. Dept. Agr.* ARS-71-27, 22 pp.

*Research supported by PL 480 funds.

1963. Chemically modified oil products and industrial uses. A list of publications and patents for 1962. North. Util. Res. Devlpmt. Div., Agr. Res. Serv., U.S. Dept. Agr. ARS-71-27-1, 4 pp.

1962. Edible soybean oil. A list of publications and patents, 1936-1961, Northern Regional Research Laboratory. North. Util. Res. Devlpmt. Div., Agr. Res. Serv., U.S. Dept. Agr. ARS-71-25, 15 pp.

1963. Edible soybean oil. A list of publications for 1962. North. Util. Res. Devlpmt. Div., Agr. Res. Serv., U.S. Dept. Agr. ARS-71-25-1, 2 pp.

1962. Processing oilseeds, oil, and meal. A list of publications and patents, 1936-1961, Northern Regional Research Laboratory. North. Util. Res. Devlpmt. Div., Agr. Res. Serv., U.S. Dept. Agr. ARS-71-24, 11 pp.

1963. Processing oilseeds, oil, and meal. A list of publications for 1962. North. Util. Res. Devlpmt. Div., Agr. Res. Serv., U.S. Dept. Agr. ARS-71-24-1, 1 page.

AREA NO. 8: SOYBEANS
FEED, FOOD AND INDUSTRIAL USES FOR MEAL AND PROTEIN

Problem. Production of soybeans continues to increase rapidly and exceeded 670 million bushels in 1962. For profitable disposition, now and in the future, of the growing supplies of meal from U. S. soybeans, improved feed products and new food and industrial uses are needed. Europe is developing a mixed-feed industry that needs high-protein concentrates. This market could approach that in the U. S. which uses high-protein meal from 450 million bushels of soybeans. For U. S. soybeans to achieve the maximum share of this market, more fundamental information is needed on the proteins and other nutritionally important constituents of soybeans and on the effects of processing on these components. Such information should make possible the production of feeds from soybeans having maximum feeding value that would meet the requirements of foreign markets as well as help maintain or increase the use of soybean feeds in the U. S.

U. S. soybeans could play a dominant role in alleviating the world shortage of dietary protein if more information were available on utilizing soybeans and soybean meal, flour, protein and protein concentrates in food products tailored to meet the nutritional and palatability requirements of foreign markets. That the possibilities are very real for increased utilization of soybeans in foreign foods is indicated by recent work of the Department that showed how to use U. S. soybeans for Japanese foods. The result of this work was that a market for selected soybeans for Japan was opened that now exceeds one million bushels per year. If U. S. soybeans are to achieve the maximum share of foreign food markets, basic information on nutritionally important components and effects of processing on these components will be needed. In addition, better knowledge will be required of how to use soybean protein products in foodstuffs that will be acceptable abroad.

Opportunities also exist for developing new or improved products from soybean meal and protein for industrial use in adhesives, surfactants, emulsifiers, viscosity improvers, and related products. For example, a successful method for stabilizing soybean protein against microbial attack could result in regaining the market for soybean protein as viscosity improvers for water-base paints or as emulsifiers for asphalt. This potential could be realized if more basic information were available on the physical and chemical properties and chemical reactions of components of soybean meal.

USDA PROGRAM

The Department has a continuing long-range program involving organic and physical chemists and biochemists engaged in basic research on the characterization of components of soybean meal and protein and application of

the knowledge gained to solution of problems encountered in processing and utilization of soybean meal and protein.

The Federal scientific effort on utilization of soybeans and soybean meal and protein totals 11.2 professional man-years. Of this number 4.2 are devoted to chemical composition and physical properties and 7.0 to food products.

Research at Peoria, Illinois, on chemical composition and physical properties (4.2 professional man-years) involves basic studies on isolation and characterization of components of whey proteins and acid-precipitated proteins.

Research at Peoria, Illinois, on food products (6.6 professional man-years) is devoted to development of information on specially processed soybean products pertinent to their use in high-protein foods for foreign markets. A research contract (.4 professional man-year) at the University of Illinois, Urbana, Illinois, is concerned with investigation of factors possibly present in soybeans that could cause digestive disturbances.

The current program at Peoria, Illinois, does not include research on industrial or feed products. During the reporting period the project covering research on stabilization of soybean protein against microbial attack, which had been in abeyance, was discontinued.

The Department also sponsors research on utilization of soybeans conducted by foreign institutions under grants of PL 480 funds. Research on chemical composition and physical properties involves grants to the University of Edinburgh, Scotland, for investigations on polysaccharides of soybeans (4 years, 1960-1964); to the Weizmann Institute of Science, Rehovot, Israel, for research on complexes between soybean protein and other components of the meal (5 years, 1961-1966); and to Kagawa University, Kagawa, Japan, for a chromatographic study of soybean sugars and oligosaccharides (3 years, 1963-1966).

Research on food products involves grants to the National Institute of Nutrition, Rome, Italy, for studies on use of soybean protein in pasta (4 years, 1960-1964); the Central Miso Institute, Tokyo, Japan, for studies on miso made from dehulled soybean grits (3 years, 1962-1965); Bar-Ilan University, Ramat Gan, Israel, for studies on miso-type food products for use in Israel (3 years, 1962-1965); Israel Institute of Technology, Haifa, Israel, for evaluation of the quality of isolated soybean protein for use in Israeli foods (4 years, 1962-1966); Japan Tofu Association, Tokyo, Japan, for studies on the use of U. S. soybeans for making tofu (2 years, 1963-1965); Academica Sinica, Nankong, Taiwan, for investigation on preparing Chinese cheese from soybeans (5 years, 1963-1968); and Noda Institute for Scientific Research, Noda-chi, Chiba-ken, Japan, for studies on improved strains of Saccharomyces rouxii for making shoyu and miso (5 years, 1963-1968). Also, a contract, financed with PL 480 funds, has been placed with

the Japan Shoyu Institute, Tokyo, Japan, for comparative evaluations of soy sauces prepared from Japanese and U. S. soybeans (2.5 years, 1961-1963).

Research on feed products involves a grant to the Hebrew University, Rehovot, Israel, for basic studies on soybean saponins (5 years, 1961-1966).

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition and Physical Properties

1. Acid-precipitated protein. Fractionation of material extracted by 86-percent ethanol from dialyzed, acid-precipitated protein indicated presence of over 25 components. Those identified so far are triglyceride, which comprises about one-third of the material, and smaller amounts of phosphatidyl choline, phosphatidyl ethanol amine and genistein. Studies on denaturation of isolated protein with isopropanol showed that 40 percent isopropanol was most effective. Rate of denaturation increased with temperature but was unaffected by pH over the range 4.5 to 7.5. Both the 11S and 7S components were insolubilized by isopropanol; the 7S component was more sensitive to denaturation than the 11S. Other studies of 11S component of soybean protein showed that the apparent molecular weight decreases with increasing protein concentration.

2. Whey proteins. Soybean trypsin inhibitors A_1 and A_2 caused growth inhibition and pancreatic hypertrophy. These results are similar to those obtained earlier with purified Kunitz inhibitor. Rat bioassays showed that in a 10-percent-casein diet 0.5 percent of crystalline trypsin inhibitor caused nearly maximum growth inhibition and pancreatic hypertrophy, whereas in a 14-percent-casein diet 0.6 percent of inhibitor had no effect on growth but did produce maximum pancreatic hypertrophy. Feeding tests were performed at the Western Division.

The indication that an increased level of protein in the diet can overcome growth inhibition by trypsin inhibitor may explain many of the contradictory observations and conclusions hitherto associated with studies of the nutritive value of raw soybean meal. New opportunities for development of improved soybean meals may result from further study of this phenomenon.

3. Soybean polysaccharides. Under a PL 480 grant to the University of Edinburgh, Scotland, two galactomannans, two pectic acids, and two hemi-celluloses have been isolated from soybean hulls and their structures are being determined. Further fractionation and isolation of polysaccharides from hulls and from soybean cotyledon meal are underway. The rate of progress indicates that the major polysaccharides in soybeans will be isolated and characterized during the course of this project.

4. Complexes of soybean protein with other meal constituents. The Weizmann Institute of Science, Rehovot, Israel, has fractionated soybean proteins using DEAE-cellulose and calcium phosphate chromatographic

procedures. The protein fractions were examined for antiproteolytic, hemagglutinating, and β -amylase activities. Ultraviolet spectral studies indicated the presence of nucleic acids in two of the protein fractions. This work is being performed under a PL 480 grant.

B. Food Products

1. Tempeh. Tempeh is an Indonesian food product obtained by fermentation of soybeans with strains of Rhizopus mold, of which research at the Northern Division showed R. oligosporus to be the best producing strain.

Primitive methods used in Indonesia to make tempeh have been adapted to a rapid, simple process. In this modern process, pure strains of the tempeh-producing mold are used, and the soybeans are fermented in plastic packages. The traditional method uses mixed strains of mold for fermentation and banana leaves for packaging.

Feeding tests conducted at the Western Division suggested that tempeh made by short-term fermentation with Rhizopus may effect a slight decrease in protein efficiency with rats when compared to full-fat soybean flour. Amino acid analysis is not precise enough to show a loss of any of the essential amino acids in a 24-hour fermentation. The rat-feeding studies showed that protein efficiency ratio for tempeh varied from 2.35 to 2.64 compared to 2.62 to 2.78 for toasted full-fat soybean meal. Only the lowest value for tempeh represents a statistically significant decrease in nutritive value. Degree of fermentation may be responsible for the variable protein efficiency ratio of tempeh. Other work elsewhere with tempeh fermented for longer periods has shown that a loss of methionine does occur. Unidentified factors, perhaps vitamins or minerals, could be involved. The nutritional value of tempeh made by the Northern Division process appears, however, to be adequate to justify use of the process in any country.

Soybeans were found to contain a water-soluble and heat-stable substance that inhibits tempeh fermentation. This inhibitor causes no problem if water used in soaking, cooking and washing the soybeans is removed. These operations result, however, in losses amounting to about 25 percent of the weight of the beans. Studies are in progress to isolate and identify the inhibitor and to find means for its inactivation or elimination. An assay was developed for following activity of the soybean tempeh mold inhibitor during fractionation and isolation studies.

2. Full-fat soybean flour. UNICEF cooperative program. Three large-scale pilot runs at Wenger Mixer Manufacturing Company showed that a product having nutritional value comparable to commercially toasted soybean flakes could be obtained by the expansion cooking process and provided detailed information on a number of critical operating variables. Twelve tons of dehulled soybeans were flaked by Loma Linda Food Company and processed by Wenger in 12 separate tests. Samples from each test were converted to flour

at the Northern Division. Proximate analysis met specifications of commercial full-fat soybean flours. Rat bioassays and chick feeding tests indicated high biological value comparable to that of commercial toasted soybean meal. Preliminary tests of oxidative stability of the flour have given encouraging results. Results continue to be favorable to the future development of the expansion cooking process as a rapid, simple method for small-scale processing suitable for use overseas. Furthermore, important new information correlating processing conditions with biological value is being obtained. UNICEF plans test feeding of the full-fat soybean flours to 3,000 children in Taiwan in comparison with commercial soybean food products.

3. Flavor and nutritive qualities of soybean food products. Treatment with steam for 4 minutes was found to remove most of the raw beany flavor from soybean flakes; grits and whole beans required a slightly longer time. Prolonged treatment did not remove all flavor from full-fat flour. Use of ultrahigh frequency (UHF) radio waves on whole soybeans removed the beany flavor in 3 to 4 minutes. During the treatment the beans swelled, hulls were loosened, and the texture became friable. Residual flavor resembled that of peanuts. Urease activity was comparable to that of flakes steamed 6 to 10 minutes. Puffing beans with steam at 220 p.s.i. after 25 seconds in a puffing "gun" was sufficient to remove flavor.

4. Comparison of U. S. and Japanese soybeans for soy sauce. Ordinary U. S. and Japanese soybeans have been compared on a commercial scale in the making of soy sauce (shoyu) at 13 plants in Japan. Eleven of the 13 report that U. S. soybeans are somewhat superior to Japanese soybeans for making shoyu. They report specifically that the nitrogen utilization of U. S. beans is several percent higher than that for the Japanese beans. These results are very significant in promoting the export of soybeans to Japan. This research is being conducted by the Japan Shoyu Institute, Tokyo, Japan, under a PL 480 contract.

5. Quality of isolated protein for use in Israeli-type foods. Initial studies under a PL 480 grant at the Israel Institute of Technology, Haifa, Israel, indicate that untoasted soybean meal produced commercially in Israel has a very low nitrogen solubility index (NSI). As a result, yields of isolated protein were low. Factors responsible for the low NSI are being investigated.

6. Studies on miso. Under a PL 480 grant to the Central Miso Institute, Tokyo, Japan, 11 Japanese and U. S. varieties of soybeans have been studied in the preparation of miso. The U. S. soybeans had lower moisture, higher oil and less carbohydrate content than the Japanese beans, but the protein contents were almost identical. The U. S. and Japanese beans, after soaking and steaming, took up about the same amount of water and had the same hardness. After digestion with takadiastase, U. S. beans had slightly higher water-soluble nitrogen and amino nitrogen and less reducing sugar than Japanese soybeans.

In studies in progress under a PL 480 grant at Bar-Ilan University, Ramat Gan, Israel, miso has been prepared satisfactorily from soybeans in preliminary experiments, and work is underway on making miso from defatted soybean flakes. One difficulty encountered with this new substrate is the growth of the koji mold on the fermenting mass. The flakes also absorb much more water than the whole soybean and, consequently, the miso from flakes has a higher moisture content. However, samples below the mold growth are reported to be good miso.

C. Feed Products

1. Effects of saponin on nutritional quality of soybean feeds and foods. Preliminary data reported last year indicated that autoclaving soybean meal according to commercial practice destroyed most of the hemolytic activity of saponins in the meal. Further studies appear to refute the early results. Furthermore, autoclaved saponin extracts and saponin extracts from autoclaved meal inhibit the proteolysis of trypsin. Studies are underway to assess the importance of this finding on nutritional value of the meal. This research is being conducted by the Hebrew University, Rehovot, Israel, under a PL 480 grant.

PUBLICATIONS AND PATENTS REPORTING RESULTS OF USDA AND COOPERATIVE RESEARCH

Chemical Composition and Physical Properties

Eldridge, A. C., Nash, A. M., and Smith, A. K. Dec. 18, 1962. Soybean whey protein-polysaccharide complex. U. S. Patent 3,069,327.

Rackis, J. J., Sasame, H. A., Mann, R. K., Anderson, R. L., and Smith, A. K. 1962. Soybean trypsin inhibitors: Isolation, purification and physical properties. Arch. Biochem. Biophys. 98(3), pp. 471-478.

Rackis, J. J., and Smith, A. K. Aug. 14, 1962. Soybean trypsin inhibitors and method of isolating the same. U. S. Patent 3,049,530.

Smith, A. K. 1962. Soybean proteins--extraction, isolation, and properties. Proc. Symp. What's New in Products from the Soybean, sponsored by Midwest Section, Am. Assoc. Cereal Chem., April 3, 1961, Chicago, Ill., pp. 4-17.

Smith, A. K., Nash, A. M., Eldridge, A. C., and Wolf, W. J. 1962. Recovery of soybean whey protein with edible gums and detergents. J. Agr. Food Chem. 10(4), pp. 302-304.

Wolf, W. J., Babcock, G. E., and Smith, A. K. 1962. Purification and stability studies of the 11S component of soybean proteins. Arch. Biochem. Biophys. 99(2), pp. 265-274.

Food Products

Shibasaki, K.,¹ and Hesseltine, C. W. (¹Tohoku University, Sendai, Japan). 1962. Miso fermentation. Econ. Bot. 16(3), pp. 180-195.

General

Senti, F. R. 1962. Research implications and problems in using soybean products in human foods. *Soybean News* 13(2), pp. 5-6.

Senti, F. R. 1963. Current status of soybean utilization research under PL 480. *Soybean Dig.* 23(7), pp. 28, 30-34.

Senti, F. R. 1963. Soybeans--their future as a food and feed crop. *Soybean Dig.* 23(3), pp. 16-20.

1962. Composition of oilseeds. A list of publications, 1936-1961, Northern Regional Research Laboratory. North. Util. Res. Devlpmt. Div., Agr. Res. Serv., U. S. Dept. Agr. ARS-71-23, 17 pp.

1963. Composition of oilseeds. A list of publications and patents for 1962. North. Util. Res. Devlpmt. Div., Agr. Res. Serv., U.S. Dept. Agr. ARS-71-23-1, 3 pp.

1962. Edible soybean protein products. A list of publications and patents, 1936-1961, Northern Regional Research Laboratory. North. Util. Res. Devlpmt. Div., Agr. Res. Serv., U.S. Dept. Agr. ARS-71-26, 9 pp.

1963. Edible soybean protein products. A list of publications and patents for 1962. North. Util. Res. Devlpmt. Div., Agr. Res. Serv., U.S. Dept. Agr. ARS-71-26-1, 2 pp.

1962. Industrial uses of proteins. A list of publications and patents, 1936-1961, Northern Regional Research Laboratory. North. Util. Res. Devlpmt. Div., Agr. Res. Serv., U.S. Dept. Agr. ARS-71-28, 11 pp.

1963. Industrial uses of proteins. A list of publications for 1962. North. Util. Res. Devlpmt. Div., Agr. Res. Serv., U.S. Dept. Agr. ARS-71-28-1, 1 page.

1962. Soybean products for protein in human foods. Proc. Conf. sponsored by U.S. Dept. Agr., United Nations Children's Fund, and Soybean Council of America, Peoria, Illinois, Sept. 13-15, 1961. North. Util. Res. Devlpmt. Div., Agr. Res. Serv., U.S. Dept. Agr. ARS-71-22, 242 pp.

AREA NO. 9: REPLACEMENT CROPS
UTILIZATION POTENTIAL - NORTHERN REGION

Problem. Farmers could achieve more economic use of their land if new and profitable crops were available for their choice that would have different end-use patterns from those presently grown. For example, it would be advantageous to develop a new oilseed crop yielding unique fatty acids that could find industrial use in applications for which acids from presently available domestic oilseed crops are unsuitable. To develop a new crop, three basic steps are involved: (1) survey of wild plants, in cooperation with plant scientists, to identify those having both potentially valuable components and promising agronomic potential for use in the United States; (2) detailed physical and chemical characterization of components of interest to obtain clues to likely end uses; (3) selection of the most promising species followed by additional utilization research to explore uses and demonstrate industrial potential and by additional agronomic research to establish proper cultural practices and to select the best strains and varieties. Only after these steps have been successfully accomplished can a proposed new crop be offered to agriculture and industry for introduction and development. Obviously, a program of this type is a long-range one. Yet, whether the future of agriculture involves conditions of surplus, of greater emphasis on foods and feeds, or of necessity for greater national self-sufficiency, the nation will benefit from availability of optimum, practical crop plants to serve its needs.

To achieve the objective, survey and characterization work needs to be greatly increased, since the greater the number of species examined, the greater will be the opportunities for finding plants meeting the criteria of high utilization and agronomic potentials. Work of the Department has already revealed several promising sources of new potentially valuable water-soluble gums, pulp fibers, and oils containing unique fatty acids such as hydroxy-unsaturated acids, capric acid, epoxidized acids, and unusual long-chain fatty acids. In order to demonstrate the potential of these new materials, further work is required on their physical and chemical properties and reactions, on processing to obtain maximum recovery from source plants, and on byproducts from processing, such as oilseed meals.

USDA PROGRAM

The Department conducts a long-range continuing program of research involving analytical and organic chemists and chemical engineers engaged in examination of uncultivated plants to find unusual and potentially useful components and in detailed characterization and evaluation studies of selected components that have the greatest industrial potential and that are obtainable from agronomically promising plants. Plants or seeds for this program are obtained by cooperation with Crops Research Division which procures material from domestic and foreign sources by means of

collecting trips or from experimental plantings. Materials from abroad are also made available through Crops Research Division PL 480 projects providing for collecting activities by foreign investigators. All seeds and plants are submitted to a broad chemical-screening program to identify sources of unusual and potentially useful components such as oils, fibers, gums, amino acids and proteins. Components of interest from plants rated by Crops Research Division as having a reasonable agronomic potential for the United States are characterized to obtain clues to areas of utilization of probable interest to industry. On the basis of the results, plants having the highest agronomic potential and containing components of greatest potential industrial value are selected for more intensive utilization research. This utilization research is divided among the four Utilization Research and Development Divisions.

The Federal scientific effort devoted to research on replacement crops at Peoria, Illinois, totals 23.1 professional man-years. Of this number, 14.5 are concerned with chemical composition and physical properties; 7.8 with industrial utilization of new oilseeds; and .8 with industrial utilization of new gum and fiber plants.

Research at Peoria, Illinois, on chemical composition and physical properties (14.2 professional man-years) involves conduct of the program on screening uncultivated plants for unusual and potentially useful oils, fibers, gums, amino acids and other components; organic chemical characterization of selected fractions and components, especially new oils fatty acids; and studies on properties of new plant fibers. A research contract (.3 professional man-year) is in effect with Montana State College, Bozeman, Montana, providing for screening and analysis of seed oils of Brassica (mustard) and related genera to identify species having greatest erucic acid content and agronomic potential.

Research at Peoria, Illinois, on industrial utilization of new oilseeds (7.8 professional man-years) involves studies on processing of erucic acid oilseeds to obtain oil and meal and investigations on utilization of erucic acid and its derivatives. During the reporting period, research was completed on development of a method for processing mustard seed to bland oil and to byproduct meal suitable for livestock feeding.

Research at Peoria, Illinois, on industrial utilization of new gum and fiber plants (.8 professional man-year) is concerned with development of methods for recovery of gums from plants; with evaluation of plant gums in industrial application; and with studies on pulping new fiber plants and evaluation of the pulp in paper, structural boards and related products. During the reporting period research on utilization of Crotalaria intermedia gum was completed.

The Department also sponsors research in this area conducted by a foreign institution under a grant of PL 480 funds (5 years, 1962-1967). This work, performed by the Institute of General Chemistry, Warsaw, Poland, is

concerned with determination of glyceride structure of erucic acid oils and is under the subheading chemical composition and physical properties.

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition and Physical Properties

1. Screening for new industrial oils. Since the last report, 690 additional samples of seeds were screened for new oils of potential interest. Oil from Crepis foetida, a species from Turkey, contained 60 percent of an acid previously observed in lower concentration in Helichrysum bracteatum. Alkali isomerization indicated 88 percent "apparent linolenic" acid while chromatography showed none. This discrepancy is explained by structural characteristics of the unknown acid (see item 2 below). Two Cuphea species contained 70 percent of caprylic acid and 20 percent of capric acid in contrast to C. llavea and C. ignea (85 percent capric acid) and C. carthagrenensis (57 percent lauric and 18 percent capric acids). Six sunflower introductions from Russia contained more oil and protein than domestic samples but linoleic acid contents of the oils were in the same range (30-54 percent).

Special screening studies of the 7 available known Limnanthes species and 30 species related to Dimorphotheca were completed. In all of the Limnanthes oils, at least 95 percent of the acids are C-20 and C-22 acids with the C-20 acid (5-eicosenoic acid) predominating (52 to 77 percent). Dimorphecolic acid (28 to 75 percent) was found in all 6 of the Dimorphotheca and Castalis species tested and in 5 of the Osteospermum species. The remaining 19 species (Osteospermum, Calendula, Chrysanthemoides) contained 14 to 60 percent of a conjugated trienoic acid but little or no dimorphecolic acid. Oil from five samples of crambe seed grown in Nebraska contained 54 to 59 percent of erucic acid.

Contract research at Montana State College revealed that seed oil of an Iberis species was as high in erucic acid content as crambe oil; however, oil yield and crop potential are inferior to those of crambe. On the basis of compositional data obtained so far on Brassicas, erucic acid content of some lines is independent of environment and may be controlled by one gene. Addition of a second gene apparently results in a level of erucic acid that is influenced by environment.

2. Characterization of new seed oils and components. The unusual acid of Crepis mentioned in item 1 has been characterized as cis-9-octadecen-12-ynoic acid. In the presence of alkali this acid rearranges to conjugated trienoic acid. Cis-11-eicosenoic acid was shown to be present in the seed oils of Marshallia caespitosa, Alyssum maritimum and Selenia grandis to the extent of 44, 42, and 58 percent, respectively. About 16 percent of the fatty acids of Leonotis nepetifolia seed oil is an optically active unsaturated acid that may contain an allenic grouping and chain branching and that is convertible to an optically active saturated acid.

Studies on the glyceride structure of erucic acid oils have been initiated by the Institute of General Chemistry, Warsaw, Poland, under a PL 480 grant.

3. Characterization of components of crambe and other oilseed meals. Based on study of a limited number of species, an analytical method for determining total thioglucoside in seed meals has been developed. It depends upon estimation of sulfate ion formed by hydrolysis of thioglucoside with the enzyme myrosinase. Although sulfate formation was unaffected by pH over the range studied, the amount of oxazolidinethione was strongly pH dependent. Total thioglucoside in hexane-extracted meal ranged from 7.1 to 8.4 percent for 5 samples of crambe seed. A crystalline oxazolidinethione and a crystalline acetate of a thioglucoside have been isolated from crambe meal and are being characterized. From an 80 percent aqueous acetone extract of crambe meal, 1 to 2 percent of a crystalline prolamine protein has been isolated. The residue insoluble in aqueous acetone amounts to 75 percent of the starting material and contains 56 percent of protein high in lysine and methionine. The lipase enzyme system of crambe was found to be inactive at 12 to 15 percent moisture content or less at room temperature.

Continued studies on amino acid composition of seed meals (39 species of 16 plant families) revealed variability within previously demonstrated limits.

The crystalline protein isolated from crambe is the first prolamine that to our knowledge has been obtained in crystalline form. This accomplishment has considerable basic chemical importance. Successful isolation of crystalline thioglucoside derivative and oxazolidinethione from crambe will greatly assist studies of their properties and reactions and of methods for their destruction or inactivation. The advance in knowledge of myrosinase activity and the products formed, and the development of an analytical procedure for determining total thioglucosides in a seed meal, will be extremely valuable in developing and controlling improved methods for processing crambe. The analytical method is believed to be the first of its kind; it should, therefore, have value generally in the study of thioglucosides in plants.

4. Screening for new seed mucilages. Of 108 new species surveyed, six contained more than 20 percent of water-soluble mucilage. One is a legume, Astragalus hamosus, collected in Turkey and rated as having a good crop potential. Another four legumes, collected in Mexico, have fair to good crop potential. The remaining species, Sphinctospermum constrictum, another legume from Mexico, was rated excellent agronomically.

5. Screening for new pulp fiber plants. Data on screening studies of 172 not previously studied plant species, on 128 sorghum species samples and on statistical analysis of results with 11 selected monocots and

dicots is being summarized for publication. To complete the studies, percent pith is being determined for the sorghum samples. For the 114 samples so far analyzed, "apparent pith" ranged from 9 to 55 percent. In connection with the study of dicots and monocots, data to establish approximately 2,700 correlations are now being processed by Biometrical Services.

B. Industrial Utilization of New Oilseeds

1. Processing crambe seed. A dehulling process for crambe has been developed that increases protein content of the meal from 40 to 50 percent (moisture-free basis). Application of the enzymatic hydrolysis process to crambe yielded meal containing very low levels of isothiocyanates and oxazolidinethiones. Sulfur balances indicated, however, that 92 percent of the original sulfur was still present as inorganic sulfate, thioamino acids and uncharacterized compounds. Whereas feeding raw crambe meal to rats caused death in 4 weeks, detoxification by the enzymatic hydrolysis process gave meals which, when fed at the Western Division to rats in amounts up to 30 percent of the ration, gave growth rates up to 80 to 90 percent of that of the addis control diet. Subsequent tests showed that hydrolysis may not have been complete during processing of these meals. In view of these results and those from the basic studies on components of crambe meal (see preceding item A-3), prospects appear good for eventual development of a palatable nutritious feed meal from crambe.

2. Studies on utilization of erucic acid. Oxidative stability tests showed the following induction periods: for crude and refined crambe oil, 186 and 53-115 hours; for crude and refined soybean oil, 129 and 43 hours. Directed interesterification and fractionation of crambe oil yielded a fraction containing increased amounts of erucic acid.

In ozonization of erucic acid, conversions of over 90 percent and a yield of 98 percent pure brassyllic acid amounting to 70 percent of theory were obtained. Several diesters of brassyllic acid, tested at the Eastern Division, showed excellent compatibility and low temperature flexibility as plasticizers for poly(vinyl chloride). A second series of 14 aliphatic diesters of brassyllic acid was prepared for evaluation at the Eastern Division as vinyl plasticizers. Tests at the Western Division indicated that brassylloyl chloride was comparable to sebacoyl chloride in the WURLAN treatment of wool. Other derivatives prepared for study include divinyl brassylate, polyoxyethylene brassylate esters, and the ester of brassyllic acid and crambe alcohols.

C. Industrial Utilization of New Fiber Plants

1. Kenaf for pulp and paper. By adaptation of milling and classification techniques, and by other procedural changes, improvements have been made in mechanical pulping of kenaf to achieve more closely controlled fiber size, fiber size combinations, degree of hydration and other characteristics.

Mechanical-type pulps were obtained from kenaf in yields of 85 to over 90 percent, while yields of bleached chemical pulps were about 40 to 45 percent. Newsprint-type papers prepared from blends (30:70 parts by weight) of chemical and mechanical kenaf pulps exceeded in most physical properties the average of 50 commercial newsprint papers. All properties were within the acceptable to superior range with the possible exception of brightness, which was 90 percent of the commercial average. Two kenaf mechanical pulps prepared at the Northern Division were evaluated by the Hillenbrand Industries for resin-bonded hardboard. Strength properties were comparable to those of similar hardboards made from wood fiber. One boxcar (1,000 bags) of chopped kenaf from the planting at Batesville, Indiana, has been furnished to a major papermaking company for large-scale pulping studies.

Demonstration that kenaf mechanical pulps can be used as components of good quality newsprint and resin-bonded hardboards indicates the versatility of this pulp source. It further shows good potential, justifying further studies to optimize process efficiency and product quality.

PUBLICATIONS AND PATENTS REPORTING RESULTS
OF USDA AND COOPERATIVE RESEARCH

Chemical Composition and Physical Properties

Bagby, M. O., Smith, C. R., Jr., Mikolajczak, K. L., and Wolff, I. A. 1962. Thalictrum polycarpum fatty acids--a new class of fatty acids from vegetable seed oils. *Biochemistry* 1(4), pp. 632-639.

Earle, F. R. 1962. Determination of protein. A selected annotated bibliography. In "Cost Reductions Through In-Plant Production Controls and Other Reports," Proc. 1962 Feed Production School, sponsored by Midwest Feed Manufacturers' Assoc., Kansas City, Mo., Nov. 12-14, 1962, pp. 105-107.

Earle, F. R., and Jones, Q.¹ (¹USDA Crops Research Div., Beltsville, Md.). 1962. Analyses of seed samples from 113 plant families. *Econ. Botany* 16(4), pp. 221-250.

Earle, F. R., Wolff, I. A., Glass, C. A., and Jones, Q.¹ (¹USDA Crops Research Div., Beltsville, Md.). 1962. Search for new industrial oils. VII. *J. Am. Oil Chemists' Soc.* 39(9), pp. 381-383.

Miller, R. W., VanEtten, C. H., McGrew, C., Wolff, I. A., and Jones, Q.¹ (¹USDA Crops Research Div., Beltsville, Md.). 1962. Amino acid composition of seed meals from forty-one species of Cruciferae. *J. Agr. Food Chem.* 10(5), pp. 426-430.

Miwa, T. K., Earle, F. R., Miwa, G. C., and Wolff, I. A. 1963. Fatty acid composition of maturing Vernonia anthelminthica (L.) Willd. seeds. Dihydroxyoleic acid--a possible precursor of epoxyoleic acid. *J. Am. Oil Chemists' Soc.* 40(6), pp. 225-229.

Smith, C. R., Jr., Wilson, T. L., Bates, R. B.,¹ and Scholfield, C. R. (¹University of Illinois, Urbana). 1962. Densipolic acid: A unique hydroxydienoid acid from Lesquerella densipila seed oil. *J. Org. Chem.* 27(9), pp. 3112-3117.

VanEtten, C. H., and Miller, R. W. 1963. The neuroactive factor α - γ -diaminobutyric acid in angiospermous seeds. Econ. Botany 17(2), pp. 108-109.

VanEtten, C. H., Rackis, J. J., Miller, R. W., and Smith, A. K. 1963. Amino acid composition of safflower kernels, kernel proteins, and hulls, and solubility of kernel nitrogen. J. Agr. Food Chem. 11(2), pp. 137-139.

Industrial Utilization of New Oilseeds

Miwa, T. K., and Wolff, I. A. 1962. Fatty acids, fatty alcohols, and wax esters from Limnanthes douglasii (Meadowfoam) seed oil. J. Am. Oil Chemists' Soc. 39(7), pp. 320-322.

Mustakas, G. C., Kirk, L. D., and Griffin, E. L., Jr. 1962. Mustard seed processing: Bland protein meal, bland oil, and allyl isothiocyanate as a by-product. J. Am. Oil Chemists' Soc. 39(8), pp. 372-377.

Smith, C. R., Jr., Bagby, M. O., and Wolff, I. A. Oct. 9, 1962. Method of preparing 14-hydroxy-cis-11-eicosenoic acid and ester derivatives thereof. U. S. Patent 3,057,893.

1962. Crambe. A potential new crop for industrial and feed uses. Crops Research Div. and North. Util. Res. Devlpmt. Div., Agr. Res. Serv., U.S. Dept. Agr. ARS-34-42, 19 pp.

Industrial Utilization of New Gum and Fiber Plants

Clark, T. F., Nelson, G. H., Nieschlag, H. J., and Wolff, I. A. 1962. A search for new fiber crops. V. Pulping studies on kenaf. Tappi 45(10), pp. 780-786.

Clark, T. F., and Wolff, I. A. 1962. A search for new fiber crops. VI. Kenaf and wood pulp blends. Tappi 45(10), pp. 786-789.

Trotter, W. K. (USDA Econ. Res. Serv., Peoria, Illinois). 1962. Search for new fiber crops. Part VII. An economic assessment. Tappi 45(12), pp. 915-919.

Trotter, W. K.,¹ Poats, F. J.,² and Wolff, I. A. (¹USDA Econ. Res. Serv., Peoria, Illinois; ²USDA Econ. Res. Serv., Washington, D.C.). 1962. New industrial crops--some economic considerations. Agr. Econ. Rept. No. 10, U.S. Dept. Agr., 45 pp.

General

1963. New crops. A list of publications and patents for 1962. North. Util. Res. Devlpmt. Div., Agr. Res. Serv., U.S. Dept. Agr. ARS-71-19-1, 4 pp.

AREA NO. 10: SUGARCANE
PROCESSING AND PRODUCTS - NORTHERN REGION

Problem. Quotas established by the Sugar Act effectively prevent the accumulation of surpluses by limiting production to estimated requirements at stable, low prices for sugar. Prices received by farmers of the United States and Puerto Rico for sugarcane are based upon the recoverable sugar content of the cane; and the rising costs of production and processing make imperative the more efficient recovery of increased amounts of sugar to provide adequate returns for both processors and growers. Currently recovery of 75 percent of the total sugar in the cane is considered satisfactory in Louisiana, and about 83 percent in Puerto Rico and Hawaii. Improved processing methods could increase the recoverable sugar to at least 85 percent in Louisiana and over 90 percent in other areas. The development of more efficient processing methods depends in turn upon the acquisition of adequate data on the quantitative composition of juices extracted from sugarcane, and of materials processed to recover sugar. The chemical industry provides a promising potential for the utilization of additional sugar since more than 15 billion pounds of chemical products are produced annually and sold to every section of American industry. More information is needed on the chemistry and properties of products from sugar to expand their utilization and on the application of these derivatives in the production of plastics, protective coatings, emulsifiers, detergents, and the like.

USDA PROGRAM

The major part of the Department's research program on sugarcane processing and products is maintained at the Southern Utilization Research and Development Division, New Orleans, Louisiana. At the Northern Division, Peoria, Illinois, the Department maintains a long-term continuing program involving microbiologists and biochemists engaged in basic and applied research on the fermentative conversion of sugar to industrially useful organic acids.

The Federal program at Peoria, Illinois, totals 2.5 professional man-years, all of which is devoted to new and improved products, specifically, fermentative conversion of sugar to α -ketoglutaric acid.

In addition, the Department, through the Northern Division, sponsors research in this area under a grant of PL 480 funds to the Institute of Biological Chemistry, University of Rome, Italy, for studies on the preparation and characterization of dextran derivatives (5 years, 1961-1966). This research is under the subheading, new and improved products.

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. New and Improved Products

1. Production of α -ketoglutaric acid. Conditions have been developed for reproducible conversion of glucose to α -ketoglutaric acid in 58-percent (weight/weight) yield. Pseudomonas chlororaphis (NRRL B-560) is used for the fermentation, which requires 168 hours. A 25-percent yield of 2-ketogluconic acid is also formed but no satisfactory method for converting it to α -ketoglutaric acid has been found. Conversion of glucose to 2-ketogluconic acid in yields of 85 to 95 percent (weight/weight) has been achieved in 1 to 2 days in 20-liter fermentors with Serratia marcescens.

It is doubtful if more than minor improvements in yield of α -ketoglutaric acid can be achieved by further adjustments in fermentation conditions. The process might nevertheless prove to be economical if both α -ketoglutaric and 2-ketogluconic acids could be conveniently isolated in good recovery. The process for 2-ketogluconic acid based on S. marcescens should have industrial value and deserves further developmental research and cost evaluation.

2. Studies on dextran derivatives. Both cationic and anionic derivatives of a dextran have been prepared and their interaction with selected proteins is being investigated. Discovery of soluble complex formation between these derivatives and protein assures further progress in study of reaction mechanisms and modes of binding. One entirely new derivative might find applicability based on its exceptional water-binding capacity. Other studies were concerned with the physiochemical characterization of the dextran and on the reaction of dextran with certain oxidative and hydrolytic reagents. This research is being conducted by the Institute of Biological Chemistry, University of Rome, Italy, under a PL 480 grant.

PUBLICATIONS AND PATENTS REPORTING RESULTS
OF USDA AND COOPERATIVE RESEARCH

None.

AREA NO. 11: FORAGES AND FEED
PROCESSING AND PRODUCTS - NORTHERN REGION

Problem. Fresh forage crops are the richest natural sources of a wide variety of nutrients essential to farm animals. The bulk of these crops, however, is preserved by such inefficient processes (hay making and ensiling) that 10 to 50 percent of the original dry weight and much larger amounts of certain valuable nutrients and growth-promoting factors are lost before the animal consumes the products. Dehydration is currently the only practical means for preserving a high percentage of the value of forage crops. Poultry and swine producers, aware of the value of dehydrated forage in feeds, nevertheless restrict the use of this product because of its high fiber and growth-inhibitor content. The livestock producer needs, and therefore the forage dehydrator needs to produce, feed ingredients from forages tailored to specific classes of farm animals. Intensive basic and applied utilization research are needed to develop new methods for processing forages to produce: (1) high-value, fiber-free juice or low-fiber products for nonruminant animals; (2) low-cost products, rich in fiber treated to make it highly digestible, for ruminants; and (3) a growth-stimulating supplement for ruminants, taking advantage of the presence in forages of such growth-promoting compounds as the recently discovered coumestrol. Forage products for ruminant feeding would be specifically designed for the mechanized feeding operations which will be essential for the 45-percent increase in livestock production to meet the projected 1975 requirements. Development of new processes and improved forage products would stimulate the production of large tonnages of forages as cash crops on high-value land now being used for crops currently in surplus.

USDA PROGRAM

The major part of the Department's research program on forages is maintained at the Western Utilization Research and Development Division, Albany, California. At the Northern Division, Peoria, Illinois, the Department has a short-term program involving one organic chemist engaged in research to isolate and identify the toxic component(s) of tall fescue grass responsible for a cattle disease known as "fescue foot." This research is cooperative with the Kentucky State Experiment Station which furnishes toxic and nontoxic fescue grass for chemical study and conducts bioassays of fractions and components isolated from fescue at the Northern Division. Liaison is maintained with the fescue breeding program of the Field Crops Research Branch, ARS, through the Agronomy Department of the University of Kentucky and with the Department's Pharmacology Laboratory at Albany, California.

The Federal program at Peoria, Illinois, totals 1.0 professional man-year, all of which is devoted to chemical composition and physical properties.

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition and Physical Properties

1. Fescue toxicity. Tests at Kentucky AES with their new bioassay technique indicated toxicity for crude alkaloids, aqueous residue and solids from the hexane-water interface encountered in extraction of lipids. The lipid fraction was considered nontoxic. These results contradict previous reports that only the aqueous residue showed toxicity. Evidently much additional work will be needed to standardize and to interpret properly data furnished by the new bioassay technique. It is quite possible that fescue alkaloids will prove to be implicated to a greater extent in toxicity than the earlier, cruder bioassay procedure indicated. About 0.5 ton of tall fescue hay was extracted with 80-percent ethanol. The extract was concentrated and shipped to Kentucky AES for further study.

A fraction believed to contain alkaloidal amine oxides was isolated from the aqueous residue obtained in conventional isolation of crude alkaloids. This fraction amounts to 0.17 percent of the hay or about twice the amount of the crude alkaloid fraction. Alkaloidal amine oxides have not been previously isolated from fescue. Such compounds from other plants have been reported to be toxic. It is significant that they occur in a fraction previously associated with toxicity of fescue.

PUBLICATIONS AND PATENTS REPORTING RESULTS
OF USDA AND COOPERATIVE RESEARCH

Chemical Composition and Physical Properties

Jacobson, D. R.,¹ Miller, W. M.,¹ Seath, D. M.,¹ Yates, S. G., Tookey, H. L., and Wolff, I. A. (¹University of Kentucky, Lexington). 1963. Nature of fescue toxicity and progress toward identification of the toxic entity. J. Dairy Sci. 46(5), pp. 416-422.

Yates, S. G. 1962. Toxicity of tall fescue forage: A review. Econ. Botany 16(4), pp. 295-303.

Line Project Check List -- Reporting Year July 1, 1962 to June 30, 1963

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Summary of Progress	Incl. in Area & Subheading
N1 1	Corn, wheat, and other cereal crop utilization investigations--Northern region.			
N1 1-58 (Rev. 2)	Operation and improvement of a culture collection of molds, yeasts, bacteria, and actinomycetes to provide a reservoir of authentic microorganisms for use in making antibiotics, vitamins, chemicals, polymers, assays, and identifications of importance to the national welfare.	Peoria, Ill.	Yes	5-A-1
N1 1-150	Fundamental and exploratory studies of chemical reactions of dextrose and related carbohydrates in nonaqueous solvents basic to development of new and improved industrial products and chemical raw materials from cereal grains.	Peoria, Ill.	Yes	1-A-1
N1 1-151	Comparative studies on the proteins of corn varieties having starches of widely differing amylose content to provide basic information related to the processing of such new types as high-amylose corn and to the utilization of byproduct protein therefrom.	Peoria, Ill.	Yes	4-A-3
N1 1-152	Investigation of the characteristics and classification of microorganisms of the genus <u>Absidia</u> and its relatives in the family Mucoraceae, with the exception of <u>Rhizopus</u> , as tools for use in the development of fermentations utilizing cereal crops.	Peoria, Ill.	Yes	5-A-4
N1 1-153	Exploration of fleshy fungi, algae and plant cells as fermentative agents for making useful products from cereal grains.	Peoria, Ill.	Yes	5-A-4
N1 1-155	Physical and chemical modification of amylose and new high-amylose starches for incorporation into paper products and textiles to provide a basis for expanded industrial utilization of cereal starches.*	Peoria, Ill.	Yes	4-B-2
N1 1-156(C)	Evaluation of dialdehyde starch as a tanning agent for sole leather.	Fort Lee, Va.	Yes	1-B-2
N1 1-159	Development of a process for producing in artificial culture media infective spores for use as pesticidal agents against the Japanese beetle.	Peoria, Ill.	Yes	5-C-1
N1 1-160	Laboratory studies on the transformation of dialdehyde starches to chemical derivatives and low-molecular-weight compounds having potential value for the production of industrial polymers, synthetic resins, plastics, and chemical intermediates.*	Peoria, Ill.	No	
N1 1-161	Investigation of the taxonomic relationships of bacteria in the <u>Pseudomonas fluorescens</u> species-group which are characterized by ability to oxidize glucose in unique manner, thus to facilitate the production of useful substances, particularly fermentation acids, from cereal grain.	Peoria, Ill.	Yes	5-A-2
N1 1-170	Pilot-plant investigations of conditions and methods for improving tempering and degeminating operations to increase the yield of corn oil and grits by corn dry-milling processes.*	Peoria, Ill.	Yes	3-B-1
N1 1-171(C)	Evaluation of cross-linked hypochlorite-oxidized wheat and corn starches in papermaking as a basis for the development of expanded markets for cereal grain products.	Syracuse, N.Y.	Yes	1-B-2
N1 1-172(C)	Investigation of the chemical reactions of periodate-oxidized starch (dialdehyde starch) in solutions involved in its potential practical applications as a basis for improving and enlarging its industrial utility.	St. Paul, Minn.	Yes	1-B-2

*Discontinued during reporting year.

Line Project Check List -- Reporting Year July 1, 1962 to June 30, 1963 (Cont'd.)

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Area & Subheading
N1 1-173(C)	Studies on the preparation of graft copolymers from wheat starch and a variety of non-carbohydrate monomers for conversion of starch into new polymers having properties of value for industrial applications.	Menlo Park, Calif.	Yes	2-B-3
N1 1-174	Screening for microorganisms that can be grown on cereal-based media to produce toxicants, repellents, and attractants, for use as control agents for insects.*	Peoria, Ill.	Yes	5-C-2
N1 1-175	Investigations on the carotenoid pigments of wet- and dry-milled fractions from corn, including high-amylose types, and of yellow-endosperm sorghum to provide data basic to the most effective utilization of industrial products and fractions from corn and sorghum in feeds.	Peoria, Ill.	Yes	3-A-2
N1 1-176(C)	Polymerization investigations on selected fermentation acids from cereal grains, and on selected fatty acid derivatives from linseed, soybean, and mustard oils, for conversion of these agricultural products to plastics and resins.	Tucson, Ariz.	Yes	5-B-3
N1 1-177(C)	Investigations on the interaction of wheat gluten with dialdehyde starches to improve the adhesive properties of gluten.	Ames, Iowa	Yes	2-B-5
N1 1-178	Investigations on the molecular size and state of aggregation of the amylose and amylopectin components of high-amylose corn starches to provide information basic to industrial utilization.	Peoria, Ill.	Yes	4-A-2
N1 1-179	Basic studies on the chemical structure of the amylose and amylopectin components of high-amylose corn starches to provide information needed for effective industrial utilization of these new starches.	Peoria, Ill.	Yes	4-A-2
N1 1-180(C)	Investigation of factors required by <u>Bacillus popilliae</u> and <u>Bacillus lentinorbus</u> to produce large and vigorous populations of cells in grain-based media for the mass production of spore dusts to control Japanese beetle infestations.	East Lansing, Mich.	Yes	5-C-1
N1 1-181	Studies of the effects of conditioning treatments of wheat on morphological and histochemical characteristics of milled fractions to provide information basic to the production of industrially useful fractions from wheat.	Peoria, Ill.	Yes	2-C-2
N1 1-182	A comprehensive study of sexual agglutination in yeasts as a basis for developing new yeasts and new processes for the fermentative conversion of cereal grains to new products.	Peoria, Ill.	Yes	5-A-3
N1 1-183	Exploratory studies to convert wheat flour into water-resistant, plasticlike chemical derivatives having properties suitable for industrial use in structural and insulating products and in molding compositions.	Peoria, Ill.	Yes	2-B-2
N1 1-184	Chemical conversion of wheat flour into a variety of hydrophilic polymers having a wide range of solubilities and viscosities in aqueous dispersions to meet specific industrial requirements for sizes, adhesives, and thickeners.	Peoria, Ill.	Yes	2-B-1
N1 1-185	Engineering development of a fermentation process for the production of citric acid from the wheat starch slurry of the batter process.	Peoria, Ill.	Yes	5-B-3
N1 1-186(C)	Studies on the reaction of acetylene with starch and starch-derived products as a basis for development of new products from cereal grains.	Tucson, Ariz.	Yes	1-A-2

*Discontinued during reporting year.

Line Project Check List -- Reporting Year July 1, 1962 to June 30, 1963 (Cont'd.)

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Summary of Progress	Incl in Area & Subheading
N1 1-187	Isolation and characterization of physiologically active nonprotein nitrogenous substances in corn and corn-milling products as a basis for applied processing studies to increase the use of corn.	Peoria, Ill.	Yes	3-A-1
N1 1-188	Investigations on the preparation of acetal and ketal derivatives of cereal starches to obtain starch products having increased water resistance, flexibility, and enhanced solubility in nonaqueous solvents.	Peoria, Ill.	Yes	1-B-3
N1 1-189(c)	A study of enzyme precursors and the mechanism of enzyme formation during wheat malting to provide basic information needed for the control of enzymes and enzyme action during the milling and processing of wheat.	Manhattan, Kans.	Yes	2-C-3
N1 1-190(c)	Investigations on methods for the chemical preparation and characterization of amino derivatives of cereal starches by replacement of nonglycosidic hydroxyl groups to obtain new starch products having increased stability to water, dilute acids, and alkali.	Columbus, Ohio	Yes	1-B-3
N1 1-191	Basic taxonomic studies on straight and flexuous streptomycetes of importance to the production of plant antibiotics by fermentation of cereal grains.	Peoria, Ill.	Yes	5-A-2
N1 1-192	Screening microorganisms that may be grown on cereal-based media to produce antibiotics effective against selected fungal diseases in plants, thus providing new fermentation outlets for cereal grains.	Peoria, Ill.	Yes	5-C-3
N1 1-193	Investigations on the development of chemical procedures for the production of 5-hydroxymethyl-2-furfural from cereal starch and its derivatives to provide a basis for expanded industrial utilization of cereal starches.*	Peoria, Ill.	Yes	5-D-1
N1 1-194	Search for microorganisms and a fermentative process to convert cereal grain products to xanthophylls that induce desirable pigmentation of poultry products when added to feed.	Peoria, Ill.	No	2-A-2
N1 1-195(c)	Investigations on the alkaline desulfurization of wheat gluten proteins to provide a basis for developing improved modifications of wheat products having utilization potential.	Peoria, Ill.	Yes	4-A-1
N1 1-196	Chemical investigations on amylo maize selections to guide corn breeders in the development of commercial hybrids containing high-amylase starch for industrial use.	Lafayette, Ind.	Yes	2-B-1
N1 1-197	Engineering process studies on the acid modification of wheat flour to prepare water-dispersible polymeric products and to make quantities available for product evaluation for use as sizes, adhesives, and thickeners.	Peoria, Ill.	Yes	4-B-1
N1 1-198	Engineering studies on the separation and fractionation of starch from high-amylase corn to prepare a purified corn amylose product for industrial applications and to prepare samples for laboratory studies.	Peoria, Ill.	Yes	2-A-2
N1 1-199(c)	Investigations on control of the chemical hydrolysis of cereal proteins to provide a basis for development of processes to yield polypeptides suitable for industrial uses.	Chicago, Ill.	Yes	1-B-1,4; 2-B-4
N1 1-200	Evaluation of modified cereal flours and starches as sizing agents, coating adhesives, and wet-end additives for paper in large-scale, high-speed continuous runs.	Peoria, Ill.	Yes	

*Discontinued during reporting year.

Line Project Check List -- Reporting Year July 1, 1962 to June 30, 1963 (Cont'd.)

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Area & Subheading
N1 1-201(C)	Evaluation of allylated dialdehyde starch as protective and decorative coatings, a molding resin, an adhesive, a modifier for synthetic resins, and as an electrical insulating material for expansion of the industrial use of starch.			
N1 1-202	Isolation and characterization of the toxic principle in tall fescue responsible for a cattle disease known as "fescue foot" to provide basic information for increased use of this forage.	Columbus, Ohio	Yes	1-B-2
N1 1-203(C)	Investigations on the control of a selected complex reaction of starch or related carbohydrates through controlled fluid flow dynamics and reaction conditions to provide a basis for process design and improvement leading to increased utilization of cereal grains. Chemical investigations on the molecular structure of the protein, glutenin, present in wheat gluten as a basis for increased industrial utilization of this raw material.	Peoria, Ill.	Yes	11-A-1
N1 1-204		Baltimore, Md.	Yes	1-A-3
N1 1-205(C)	Investigations on reactions of difunctional mercaptans with dextrose, starch, or related carbohydrates to form polymers having potential industrial value.	Peoria, Ill.	Yes	2-A-1
N1 1-206(C)	Evaluation of beta-carotene product from fermentation of grain-based media with <u>Blakeslea trispora</u> as vitamin A source for poultry and swine.	Tucson, Ariz.	Yes	1-A-2
N1 1-207	Investigations on the enzymatic modification of wheat flour and flour fractions by combined amylases and proteases to provide pastes of suitable viscosities for use as surface sizes and coating agents for paper.	East Lansing, Mich.	Yes	5-D-1
N1 1-208	Investigations on the conversion of cereal grains to economical and efficient soluble fermentation substrates through the action of microbial enzymes, as a basis for increasing the use of these grains by the fermentation industry.	Peoria, Ill.	Yes	5-B-2
N1 1-209	Investigations on the applicability and evaluation of chemically modified cereal grain flours and fractions as ingredients, agents, and adhesives in pulp and paperboard products as a basis for increasing industrial use of cereal grains.**	Peoria, Ill.	Yes	5-B-1
N1 1-210	Investigations on the preparation of water-dispersible hetero-derivatives of starch to obtain products having a wide range of properties for the production of adhesives, sizings, and other additives for applications in paper and related industries.**	Peoria, Ill.	Yes	1-B-1,2,4; 2-B-4
N1 1-211	Chemical reaction studies on wheat gluten and its component proteins seeking methods of modification to give properties better suited for industrial uses.**	Peoria, Ill.	Yes	1-B-3
N1 1-212	Investigations on the production of low-density plastic foams from starch-derived glucosides and related starch derivatives as a basis for increasing the industrial utilization of cereal starches.**	Peoria, Ill.	Yes	2-A-2
N1 1-213	Pilot-plant investigations on wheat dry-milling and fractionation methods for producing a wide variety of products for use in foods, feeds, and industrial products.**	Peoria, Ill.	Yes	1-B-3
N1 1-214(C)	Engineering studies on the application of pneumatic fluidization to the reactions of wheat flour with hydrogen chloride as a basis for producing sizing agents for paper.**	Ames, Iowa	Yes	2-C-1

**Initiated during reporting year.

Line Project Check List -- Reporting Year July 1, 1962 to June 30, 1963 (Cont'd.)

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in	
			Summary of Progress	Area & Subheading
N1 1-215	Investigations of the reaction of dialdehyde starch with casein, soybean protein, soy flour and dried animal blood for the production of improved wood adhesives.**	Peoria, Ill.	Yes	1-B-2
N1 1-216	Pilot-plant investigations of dry-milling operations to obtain increased yields of prime goods and oil from old and artificially dried corn and to develop a prototype corn degerminator having improved corn degerminating characteristics for production of higher quality dry-milled products.**	Peoria, Ill.	Yes	3-B-1
N1 1-217	Investigation of methods for producing microbial polysaccharides from cereal grains by continuous fermentation to reduce production costs allowing increased utilization of these potentially useful gums.**	Peoria, Ill.	Yes	5-B-4
N1 1-218(C)	Stabilization of vegetative cells of <u>Bacillus popilliae</u> grown on cereal-based media for use as an infecting agent against the Japanese beetle.**	Manhattan, Kans.	No	
N1 1-219(C)	Study of role of enzymes and enzyme activity in the formation of spores of <u>Bacillus popilliae</u> and <u>Bacillus lenticimorbus</u> as a basis for the mass production of biological insecticides by fermenting cereal grain.**	East Lansing, Mich.	No	
N1 1-220(C)	The transfer of genetic determinants of sporulation from one microorganism to another, as a basis for applied studies on the fermentative production of spore dusts for the control of Japanese beetle infestations.**	Minneapolis, Minn.	No	
N1 1-221(C)	Study of the sporulation factor produced by bacilli and its possible use in <u>Bacillus popilliae</u> and <u>Bacillus lenticimorbus</u> to develop a fermentation process for the production of spore dust to control Japanese beetle infestations.**	Urbana, Ill.	No	
N1 1-222(C)	Studies on the mechanism and kinetics of radiation and ceric ion induced grafting of cereal starches with vinyl-type monomers previously shown in exploratory studies to graft readily and efficiently with promise for new industrial outlets for starch.**	Menlo Park, Calif.	No	
N1 1-223(C)	Development studies on the semi-pilot-plant scale production of cereal grain xanthides and their use and evaluation in making corrugating board and linerboard for corrugated boxes.**	Columbus, Ohio	Yes	1-B-1
N1 1-224	Development of methods and processes to reduce viable microorganisms in wheat flour as it is produced in the mill.**	Peoria, Ill.	Yes	2-C-4
N1 1-225	Investigations on the development of new fermented wheat foods through the use of Oriental-type food molds as a basis for increasing export markets for U. S. wheats.**	Peoria, Ill.	No	
N1 1-226	Investigations on formation and properties of amino and peptide derivatives of starch to provide a basis for the development of industrially useful products from cereal grains.**	Peoria, Ill.	No	
N1 1-227	Investigations on the conversion of cereal xanthates to xanthides in physical forms suitable for use in papermaking.**	Peoria, Ill.	No	
N1 1-228(C)	Investigations on the interaction of "V" amylose with small molecules to provide basic information on the helical structure of amylose from high-amylose corn starch.**	Tempe, Ariz.	Yes	1-B-1

**Initiated during reporting year.

Line Project Check List -- Reporting Year July 1, 1962 to June 30, 1963 (Cont'd.)

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Area & Subheading
N4 2	Soybean and other oilseed utilization investigations-- Northern region.			
N4 2-64	Investigations on autoxidation in vegetable oils: Fundamental studies on the reaction of linseed and soybean oils and derived products with atmospheric oxygen to elucidate the mode of oxidation, in order to provide a basis for the discovery of methods of controlling the reaction, and the preparation of new products.*	Peoria, Ill. Los Angeles, Calif.	Yes Yes	7-A-1 6-C-1
N4 2-72(C)	Basic physical chemical studies on linseed oil emulsions and their interaction with metal oxides.	Peoria, Ill.	Yes	6-C-1
N4 2-73	Investigations on the anionic emulsification of commercial linseed oil polymers, or simple modifications thereof, and evaluation of the emulsions as vehicles for paints.	Peoria, Ill.	Yes	7-B-1
N4 2-74	Basic investigations on the selective hydrogenation of linolenic acid as a means of improving the flavor stability of edible soybean oil.	Peoria, Ill.	Yes	6-C-1
N4 2-75(C)	Preparation and evaluation of catalysts for selective hydrogenation of the linolenic acid component of soybean oil glycerides to provide basic information for increased food and industrial applications.*	Chicago, Ill.	Yes	7-B-1
N4 2-76	Stabilization of solutions and dispersions of soyflour and soybean protein against microbial deterioration for use in industrial emulsions and adhesives.*	Peoria, Ill.	No	
N4 2-77 (Rev.)	Engineering investigations on the production of cyclic fatty acids from linseed oil.	Peoria, Ill.	Yes	6-B-1
N4 2-78	Chemical and physical characterization studies on the electrophoretically and chromatographically separated proteins found in defatted soybean meal whey and on their associated nonprotein components to provide information basic to improving the industrial and feed value of whey proteins.	Peoria, Ill.	Yes	8-A-2,B-1
N4 2-79	Investigations on the major protein component of the acid-precipitated fraction of soybean proteins to gain basic information for improvement of isolated soybean protein in industrial and food products.	Peoria, Ill.	Yes	8-A-1
N4 2-80	Engineering studies on a process for converting mustard seed and closely related oilseeds into oil and detoxified meal for industrial feed products.*	Peoria, Ill.	No	
N4 2-81(C)	Preliminary investigations on potential industrial applications for aldehyde oils from soybean, linseed, and mustard oils, their derivatives, and combinations of selected aldehyde oils with cereal products.*	Columbus, Ohio	Yes	7-C-2
N4 2-82	Microbial modification of unsaturated fatty acids to produce long-chain amino acids and related valuable derivatives.*	Peoria, Ill.	No	
N4 2-83(C)	Studies on the effect of linseed oil coatings on the durability of air-entrained concrete and its resistance to freeze-thaw cycles for evaluating the use of this oil in protecting this type of concrete against deterioration.	Manhattan, Kans.	Yes	6-C-2
N4 2-84 (Rev.)	Exploratory investigations on products obtained by reacting linseed and soybean oils and their fatty acids with selected polyols and other hydrophilic reagents, and characterization of the products for utility as water-soluble paint vehicles.	Peoria, Ill.	Yes	6-C-3

*Discontinued during reporting year.

Line Project Check List -- Reporting Year July 1, 1962 to June 30, 1963 (Cont'd.)

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Summary of Progress	Incl. in Area & Subheading
N4 2-85	Investigations on the preparation, properties, and reactions of aldehyde oils obtained by the ozonolysis of soybean, linseed and erucic acid oils, as a basis for their increased industrial utilization.	Peoria, Ill.	Yes	7-C-1,2
N4 2-86	Investigations on new polymeric products from aldehydic materials obtained by the ozonization of soybean and linseed oils, as a basis for increased industrial utilization of these oils.	Peoria, Ill.	Yes	7-C-2
N4 2-87	Engineering studies on the production of aldehyde oils from soybean, linseed, and other unsaturated vegetable oils.	Peoria, Ill.	Yes	7-C-1
N4 2-88	Basic investigations on the chemical reactions of soybean and linseed oils and their fatty acids with ethylene and other commercially available olefinic compounds to produce new products having potential industrial value.**	Peoria, Ill.	Yes	6-B-1
N4 2-89	Studies on edible soybean oil: Improvements achieved by mixing soybean oil with other edible oils.**	Peoria, Ill.	Yes	7-B-2
N4 2-90(c)	Investigations on flatus caused by ingestion of soybean foods as related to the development of foreign-type foods to expand export markets.**	Urbana, Ill.	No	6-C-3
N4 2-91	Preparation of new derivatives from soybean and linseed fatty acids or oils containing vinyl groups capable of polymerizing to form new polymers and copolymers of potential industrial value in coatings and related fields.**	Peoria, Ill.	No	
N4 2-92(c)	Preparation and evaluation of heterogeneous catalysts for specificity in hydrogenation of linolenate in soybean and linseed oils to increase industrial and food applications.**	Chicago, Ill.	No	
N4 2-93(c)	Basic investigations on heterogeneous catalysts for the selective hydrogenation of linolenate in soybean oil to provide basic information for increased food applications.**	New Brunswick, New Jersey	No	
N4 2-94(c)	Investigations on the conversion of aldehyde oils to coating compositions for wood and metal as a basis for developing new industrial products from linseed and soybean oils.**	Fargo, N. Dak.	No	
N4 2-95	Investigations on improving flavor stability of edible soybean oil by concurrent ester interchange of the oil and selective extraction of trilinolenin.**	Peoria, Ill.	No	
N4 2-96	Exploratory investigations on the flavor, texture, and color of soybeans, soybean fractions and products used in foods and protein supplementation to increase the use of soybeans and soybean products in foreign markets.**	Peoria, Ill.	Yes	8-B-2,3
N4 2-97(c)	Basic studies on the organo complexes of transition metals as homogeneous catalysts for hydrogenation of soybean oil.**	Urbana, Ill.	No	
N5 1	Sugars and sirups investigations.	Peoria, Ill.	Yes	10-A-1
N5 1-69	Investigations on the fermentative production of α -ketoglutaric acid from sugar or molasses to provide new industrial outlets for these agricultural materials.	Peoria, Ill.	Yes	
N5 5	New and replacement crops utilization investigations.	Peoria, Ill.	Yes	
N5 5-15 (Rev.)	Chemical screening to determine the amount and kind of fiber and accompanying constituents in selected plants as a basis for discovering potential new domestic sources of fiber for pulp and papermaking.	Peoria, Ill.	Yes	9-A-5

**Initiated during reporting year.

Line Project Check List -- Reporting Year July 1, 1962 to June 30, 1963 (Cont'd.)

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Area & Subheading
N5 5-32 (Rev.)	Chemical survey of seed lipids from uncultivated domestic and foreign plants to discover sources containing economic amounts of industrially valuable constituents.	Peoria, Ill.	Yes	9-A-1,B-2
N5 5-33	Characterization of selected fractions and chemical components of seeds of plant species containing favorable amounts of gross constituents to obtain more specific evaluation of their potential industrial importance than is afforded by screening analyses.	Peoria, Ill.	Yes	9-A-2, ⁴
N5 5-40(C)	Chemical survey of native, introduced, or newly developed strains of <u>Brassica</u> and related genera of mustard seed to find seed oils with maximum erucic acid content, as a basis for development of new industrial oils from domestic crop sources.	Bozeman, Mont.	Yes	9-A-1
N5 5-41	Investigation of selected plants of the <u>Hibiscus</u> genus, with emphasis on kenaf and okra, to evaluate and develop fibrous products from annual plant sources having superiority or specific preferred properties for industrial use.	Peoria, Ill.	Yes	9-C-1
N5 5-44	Analytical investigations on proteins and other nitrogenous substances in meals from potential new oilseed crops, with emphasis on those bearing oils of high erucic acid content, to obtain fundamental information of value in their processing and utilization.	Peoria, Ill.	Yes	9-A-3
N5 5-47	Engineering studies on a process for converting <u>Crambe abyssinica</u> seed and closely related new oilseeds into oil and detoxified meal for evaluating the utilization potential of these new oilseed crops.**	Peoria, Ill.	Yes	9-B-1
NU P-1	Pioneering Laboratory for Microbiological Chemistry.	Peoria, Ill. Peoria, Ill.	Yes Yes	5-A-5

**Initiated during reporting year.

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Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Summary of Progress	Incl. in Area & Subheading
(10) UR-A7-(10)-7	Cereal and forage crops. Fundamental studies of enzyme systems isolated from <u>Pseudomonas</u> , to obtain information on the conversion of carbohydrates derived from cereal grains to organic acids having potential industrial value.**	Bangalore, India	No	
UR-A7-(10)-9	Collection and isolation of molds belonging to the order Mucorales, and classification of the isolates, in order to find microorganisms suitable for fermentative processes of importance in cereal grain utilization.	Allahabad, India	Yes	5-A-1
UR-A7-(10)-10	A study of survival and possible genetic change in industrially useful microorganisms subjected to lyophilization, to obtain basic information needed for the maintenance of culture collections for industrial fermentation of cereal grains.	Allahabad, India	Yes	5-A-1
UR-A7-(10)-20	Investigations on the preparation and characterization of new copolymers of cereal starch with other polysaccharides by heating mixtures in the dry state, to provide basic information for the development of new starch products suited for industrial applications.**	Ahmedabad, India	No	
UR-A7-(10)-25	Investigations on the separation of grain sorghum proteins into homogeneous protein components, to provide basic information for further characterization and application studies.**	Bangalore, India	No	
UR-A10-(10)-1	A fundamental investigation of the synthesis and chemical and physical properties of multi-chain polymers and copolymers comprised of amino acids derivable from the cereal grain protein, gliadin and zein, as a contribution to the increased utilization of cereal grains.	Rehovot, Israel	Yes	2,3-A-3
UR-A10-(10)-6	Fundamental studies on the mild oxidation of cereal grain starches by hypochlorite, hypobromite, hypochlorite-bromide mixtures, and other oxidizing agents for the determination of reaction mechanisms and the physical and chemical properties of the modified starches of importance to the production and use of this class of industrial starches.*	Jerusalem, Israel	Yes	1-A-4
UR-A10-(10)-9	Studies of the preparation of new cereal starch derivatives by the introduction of fluorine into starch and products derived therefrom, to provide a basis for the increased industrial utilization of wheat, corn, and sorghum.	Jerusalem, Israel	Yes	1-B-3
UR-E8-(10)-6	Isolation of organic phosphorus derivatives found in the yeast <u>Torulopsis utilis</u> and elucidation of their structures, to provide new basic information on the fermentation of cereal products to industrial materials by yeasts.	Helsinki, Finland	Yes	5-A-5
UR-E9-(10)-37	Fundamental investigations of the proteolysis-inhibiting effects of cereal flours and starches, and of processing methods for minimizing such effects, to provide a basis for improved quality and increased utilization of cereal products.	Paris, France	Yes	1-A-6

*Completed project.

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Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Summary of Progress	Incl. in Area & Subheading
UR-E9-(10)-40	Investigations of the zein protein of corn: Fractionation and study of rheological and physical-chemical properties, chemical composition and structure, and problems of hydration and gelification of fundamental importance to the technology and industrial utilization of corn proteins.			
UR-E9-(10)-42	A fundamental investigation of the physico-chemical alterations brought about in starches and their molecular constituents by gamma-radiations, to provide information needed for modification of starch properties and for the treatment of starch-containing products used industrially or in foods.	Paris, France	Yes	3-A-4
UR-E15-(10)-21	Investigation of the growth factor (Vitamin B ₁₃) of distillers' dried solubles through studies of methods of isolation and purification, mode of formation, and conditions of optimum production by yeast fermentation of cereal grains, to provide basic information for utilizing grains to produce this vitamin.	Paris, France	Yes	1-A-7
UR-E15-(10)-24	Investigation of aerobic fermentation processes by measurement of the effects of differences in vessel size and mechanical agitation on the concentration of dissolved oxygen, and by studies of the physicochemical properties of the foam, to obtain fundamental information needed for the increased utilization of cereal grains in fermentative processes.	Milan, Italy	Yes	5-D-2
UR-E15-(10)-25	Investigations of the reaction of cereal starch dextrans with fatty acid chlorides and fatty amines, and evaluation of the products, to provide information important to increasing the utilization of wheat, corn, and sorghum.	Rome, Italy	Yes	5-B-5
UR-E15-(10)-26	Investigation of the fermentative conversion of glucose to 5-ketogluconic acid through studies of a metabolic pathway in organisms of the <u>Acetobacter</u> genus, to obtain fundamental information for the utilization of grain products in the fermentative production of chemical intermediates.	Bologna, Italy	Yes	1-B-3
UR-E15-(10)-32	Investigations on the conformation of gluco-pyranose rings in amylose corn starches and in linear and cyclic dextrans prepared from these starches, to provide basic information for the chemical modification of starch-derived products for the development of new uses.	Milan, Italy	Yes	5-A-5
UR-E25-(10)-11	Isolation and characterization of yeasts for placement in the Culture Collection of the Agricultural Research Service, as potential agents for the conversion of farm-produced raw materials to products useful to industry and the consuming public.	Milan, Italy	Yes	1-A-5
UR-E29-(10)-36	Fundamental studies of chemical reactions for polymerizing glucose and glucose derivatives to form new high-molecular-weight compounds, as a basis for the development of new outlets for cereal grains and other starch-rich crops.	Madrid, Spain	No	
		Musselburgh, Scotland	Yes	1-B-5

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Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Summary of Progress	Incl. in Area & Subheading
UR-E29-(10)-37	Studies on the quantitative measurement of properties of wheat kernels that vary significantly during conditioning, as a basis for improved conditioning of wheat for milling by new and improved methods and increased industrial utilization of flour and milled wheat products.	St. Albans, England	Yes	2-C-5
UR-E29-(10)-39	A fundamental study of factors governing the onset of oxidative rancidity in oat products, to provide a basis for improving the quality and increasing the utilization of oats in feed and food.	St. Albans, England	No	
UR-E29-(10)-40	Investigations of the structure and properties of cereal starches--particularly corn and wheat starches--as revealed by their interaction with enzymes and other proteins, to obtain fundamental information concerning the structure and behavior of cereal starches that would be useful in starch processing.	Edgbaston, Birmingham, England	Yes	1-A-5
UR-E29-(10)-51	Investigation of sugars, their phosphate derivatives, and related compounds, as found in molds important to the fermentative conversion of cereal grains to useful products.	Newcastle-upon-Tyne, England	Yes	5-A-5
UR-S3-(10)-11 (10,40)	Preparation of cationic cereal starch derivatives for use in paper and textiles by the introduction of quaternary phosphonium and tertiary sulfonium groups into crosslinked and noncross-linked starches, to create new markets and expand old markets for starch from cereal grains. Cereal, forage crops, and oilseeds.	Rio de Janeiro, Brazil	Yes	1-B-3
UR-A11-(10,40)-10 (40)	Investigation of crosses of <u>Saccharomyces rouxii</u> isolated from the soybean fermentations, shoyu and miso, and an evaluation of their fermentative abilities in the above fermentation processes, as a basis for increasing the use of soybeans in fermented foods.** Oilseeds	Noda-shi, Chiba-ken, Japan	No	
UR-A6-(40)-1	Investigation of the various processes used in preparing Chinese cheese by the fermentation of soybean curd with <u>Mucor</u> and other fungi as a basis for increasing the foreign utilization of soybeans.**	Taipei, Taiwan	No	
UR-A7-(40)-21	Exploratory investigations of selected hydroxylated derivatives of linseed and safflower oils, to determine the feasibility of producing new industrial products from these oils.**	Hyderabad, India	No	
UR-A10-(40)-17	Fundamental investigations of complexes formed by soybean proteins with other meal constituents, to provide information for applied studies on expanded utilization of soybean oil meal.	Rehovot, Israel	Yes	8-A-4
UR-A10-(40)-18	Investigations of soybean saponins as related to the processing of petroleum ether-extracted meal for feed and to the preparation of soy foods, to provide information basic to improving the nutritional value of soybean protein products.	Rehovot, Israel	Yes	8-C-1
UR-A10-(40)-20	Laboratory investigations on miso-type food products by fermentation of soybean meal products and cereal grains for use in Israeli foods.**	Ramat Gan, Israel	Yes	8-B-6

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Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj.	Incl. in
			Summary of Progress	Area & Subheading
UR-A10-(40)-30	Investigations of the effect of processing conditions on the yield and quality of isolated soybean protein for use in Israeli-type foods, as a contribution to expanded utilization of soybeans.**			
UR-A11-(40)-1(C)	Factory experiments on comparative production of shoyu (soy sauce) from United States and Japanese soybeans, to provide data for the increased use of United States beans.	Haifa, Israel	Yes	8-B-5
UR-A11-(40)-2	Evaluation of dehulled soybean grits from United States varieties for making miso, to increase soybean utilization in Japan.	Tokyo, Japan	Yes	8-B-4
UR-A11-(40)-5	Investigation of the partial hydrogenation of soybean oil, to produce a stable liquid oil with improved properties for use in Japanese foods.	Kawagoe, Saitama-ken, Japan	Yes	8-B-6
UR-A11-(40)-11	Evaluation of United States soybean varieties as a material for producing fresh tofu to increase utilization in Japan.**	Tokyo, Japan	No	
UR-A11-(40)-12	A chromatographic study of the sugars and oligosaccharides in soybeans to provide information needed to improve processing of fat-free soybean meal for foods and feeds, thereby contributing to its expanded utilization.**	Takamatsu, Japan	No	
UR-E8-(40)-2	Investigation of continuous multi-stage countercurrent crystallization of linseed and soybean fatty acids as a practical method for producing pure unsaturated fatty acids, to provide a basis for new or improved uses of linseed and soybean oils.	Helsinki, Finland	Yes	7-C-3
UR-E15-(40)-8	An investigation of the minor constituents of linseed oil and their effect on the ability of linseed oil films to spread and adhere to surfaces, as a contribution to the expansion of markets for linseed oil.	Milan, Italy	Yes	6-A-1
UR-E15-(40)-9	Investigations of the controlled thermal polymerization of soybean and linseed oils, and of the separation and characterization of the reaction products, in order to obtain information useful in expanding and improving the industrial applicability of these oils.	Milan, Italy	Yes	6-B-2
UR-E15-(40)-10	Investigations of the effect of metallic catalysts and physical conditions on oxidative cleavage products produced in the autoxidation of polyunsaturated fatty acids, to provide basic information for applied research on the production of new industrial chemicals from soybean and linseed oils.	Milan, Italy	Yes	7-C-4
UR-E15-(40)-14	Studies of the admixture of soybean protein products with wheat flour in the manufacture of pasta (spaghetti, macaroni, etc.) to effect improvements in diets largely based upon cereals and contribute to increased utilization of soybeans.	Milan, Italy	No	
UR-E21-(40)-6	Chromatographic determination of the glyceride composition of selected erucic-acid containing oils, to provide basic information important to their utilization.**	Rome, Italy	Yes	9-A-2
		Warsaw, Poland		

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Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Area & Subheading
UR-E21-(40)-8	Investigation of the possible role of sterols in the development of flavors and odors in soybean oil through studies of sterol transformations during processing, in order to increase the utilization of soybeans in food.			
UR-E25-(40)-4	Investigations of ion exchange procedures for removing pro-oxidant metals from soybean oil, in order to contribute to expanded utilization of soybean oil through improvement of its flavor and oxidative stability during transportation, storage, and use.	Gdansk, Poland	Yes	7-A-3
UR-E25-(40)-29	Improvement of the frying quality of soybean oil through studies of the influence of processing factors and oil modifications on surface tension, interfacial tension, viscosity, and other physical properties concerned with its penetration into fried foods, to provide information for increased use in the preparation of Spanish foods.	Seville, Spain	Yes	7-A-2
UR-E29-(40)-29	Development of new uses for soybean and linseed oils through investigations of organometallic derivatives and complexes as components of protective coatings having improved properties.	Granada, Spain	Yes	7-B-3
UR-E29-(40)-49	Investigation of the reactions of unsaturated fatty acids and their derivatives in molten alkali, to discover new chemical intermediates important to the increased utilization of soybean and linseed oils.	Teddington, Middlesex, England	Yes	6-C-4
UR-E29-(40)-50	A quantitative study of the polysaccharides in fat-free soybean meal to provide information needed to improve the processing of meal for foods and feeds, thereby contributing to its expanded utilization.	London, England	Yes	7-C-4
(50) UR-E15-(50)-29	Sugar and miscellaneous crops.	Edinburgh, Scotland	Yes	8-A-3
	Preparation and characterization of dextran derivatives, and investigations of their interactions and binding, to provide basic information for increasing the utilization of sugar.	Rome, Italy	Yes	10-A-2



